MEAT OR POTATOES? REEVALUATING THE ROLE OF PLANT FOODS IN HUMAN EVOLUTION

by

Chelsea A. Leonard

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STATEMENT OF DISSERTATION APPROVAL

The dissertation of **Chelsea A. Leonard**

has been approved by the following supervisory committee members:

James F. O'Connell	, Chair	**9/29/15** Date Approved
Joan Brenner Coltrain	, Member	**9/29/15** Date Approved
Kristen Hawkes	, Member	**9/29/15** Date Approved
Amanda Henry	, Member	**9/29/15** Date Approved
Dennis O'Rourke	, Member	**9/29/15** Date Approved

and by **Leslie Knapp** , Chair/Dean of

the Department/College/School of **Anthropology**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

The role of plant foods in human evolution is largely ignored in the literature. The archaeological visibility of early hominin meat consumption, compelling ethnographic accounts of large game hunting, and recent trends in nutritional science all highlight the potential importance of animal foods in hominin diets. As a result, the nutritional contributions of plant foods remain under-explored. Ethnographic observations suggest that plants are a critical resource for mid- and low-latitude foragers. Paleoanthropologists concede that hominins were reliant on plants to some degree, but the role of meat consumption in driving changes in brain and body size in the hominin lineage are taken for granted. In the second chapter of this dissertation, I discuss human physiological requirements for specific macronutrients, and argue that meat is not a necessary dietary constituent. I present data on the plant contributions to the diet of Twe forager-horticulturalists, and examine the macronutrient and amino acid composition of common Twe plant foods. In the third chapter, I focus on the nutritional qualities of plant underground storage organs and discuss how they compare to animal foods in terms of nutritional composition and procurement effort. In the final chapter, I discuss the archaeological visibility of plant foods and assess the reliability of starch granules and phytoliths in dental calculus in recording Twe plant consumption.

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CHAPTER 1

INTRODUCTION

This dissertation discusses the contribution of plant foods to the diet of Twe forager-horticulturalists of Northwest Namibia in order to draw inferences about the importance of plant foods in early hominin diets. The Twe live in a region where large game populations have declined due to overhunting and overgrazing by livestock. As a result, the Twe are heavily reliant on maize meal supplemented by wild plant foods. This presents an interesting opportunity for research, because most recent anthropological studies of forager diets have focused on the contributions of large game hunting. This reflects a broader trend in evolutionary anthropology, where researchers assume that plant foods cannot adequately support the nutritional needs demands of increasing brain and body size in the hominin lineage.

Similarly, paleoanthropological research has focused on elucidating the timing and importance of animal foods in hominin diets, at the expense of understanding the potential role of plant foods. This is at least partially due to the archaeological invisibility of plant resources relative to animal foods. However, newer methods of plant recovery, including plant microfossils preserved in dental calculus, provide a unique opportunity to learn about specific plants consumed. This dissertation discusses the nutritional contributions of plant foods to the Twe diet and assesses the reliability of the microfossil record preserved in dental calculus by comparing information on Twe diet with the microfossil record preserved in Twe dental calculus.

Chapter 2 presents data on the macronutrient composition and amino acid content of common wild plant foods and discusses their importance in the Twe diet. The assumption that hominins needed animal foods to support increasing brain and body size receives little support from comparisons of human physiological requirements with those of our closest primate relatives. Our data suggest that the Twe are able to meet their nutritional needs with only minimal input from animal sources. In addition, we argue that some types of plant foods are more important than others in helping the Twe consume adequate amounts

of essential amino acids and fatty acids.

Chapter 3 focuses on the nutritional qualities of plant underground storage organs as well as energy expenditure during procurement. Underground storage organs are one of the few plant types that feature in hypotheses about the role of plant foods in human evolution. However, evidence for their proposed importance is equivocal, and proponents of the importance of the 'meat made us human' argument maintain that underground storage organs are a nutritionally poor resource that could not support hominin nutritional requirements. This chapter reviews published data on underground storage organ nutrient composition, and asks whether the energy spent procuring these resources negates nutritional gains.

Chapter 4 discusses the archaeological visibility of plant foods. Traditional methods of diet reconstruction include stable isotope analysis and dental macro- and microwear analysis. These methods provide general information about plant types or the qualities of foods included in the diet, but little information on specific plants consumed. In the last decade, the recovery of plant microfossils from dental calculus method has gained popularity as a way to learn about specific plants consumed, but until now, the method has not been tested in a population with a known diet. In this chapter, we compare information on Twe plant consumption with starch grains and phytoliths preserved in Twe dental calculus to determine how well this method reflects diet on both an individual and population level.

CHAPTER 2

THE ROLE OF PLANT FOODS IN HOMININ DIETS: LESSONS FROM THE TWE

2.1 Introduction

Gathered plants are an important dietary constituent for mid- and low-latitude foragers [1, 2, 3, 4, 5]. Plant foods are a predictable, low variance resource [6], and the abundance and distribution of plants was likely an important determinant of hominin diet and behavior [7]. Most evolutionary models concede that plant foods were an important part of early hominin diets, but the role of plant foods in shaping hominin morphology and behavior is overlooked in favor of animal foods. Plants do not preserve well in archaeological contexts, and while there is compelling evidence for hominin plant consumption, this evidence does not point to specific plants included in the diet. Stable isotope and dental microwear studies provide evidence for the consumption of plants with different photosynthetic pathways such as C4 grasses and sedges, or those with different physical qualities (e.g. [8, 9, 10, 11]). Plant microremains trapped in dental calculus sometimes provide information on specific plant families or genera consumed, but few studies focus on prehuman populations (e.g. [12, 13]).

In contrast, archaeological evidence for the use of animal foods is ample. In East Africa, cut marks on animal bones are frequent by 2.3 Ma [14, 15], and the early appearance of animal foods in the hominin lineage has been linked to distinctly hominin traits such as increased brain and body size in the genus *Homo* [16, 17, 18]. Meat on average contains more calories per gram than plant foods, and is a rich source of digestible protein that contains the nine amino acids necessary for human protein synthesis, as well as high concentrations of essential vitamins and minerals [19, 20]. Organ meats and marrow also contain fatty acids involved in brain growth and development [19, 21, 22]. Conventional wisdom suggests that these nutritional qualities were essental to support the metabolic demands of larger brains and bodies.

Smaller cheek teeth, thinner enamel relative to Australopiths, and greater occlusal relief in early *Homo* are cited as support for increased reliance on animal foods [23, 24, 11].

Changes in thoracic shape also indicate a reduction in overall gut size, which is consistent with a smaller, human-like colon and reduced capacity for fermentation of plant fiber. Many researchers argue that a smaller gut necessitated the consumption of energy-dense resources like meat [25, 20].

However, nowhere in the literature do we find support for the idea that meat is the only food in a foraged diet that can support human metabolic demands. In fact, a comparison with our closest living relatives reveals surprising similarities in nutritional requirements. Among the great apes, human body size is not unique. Both chimpanzees and gorillas are large bodied, and are able to meet nutritional requirements largely through consumption of plants. Gorillas are almost exclusively herbivorous, and while chimpanzees do hunt occasionally, on average, insects and meat comprise a minimal portion of their diet [26]. However, meat can be an important source of calories for chimpanzees during the dry season, especially for adult males ([27, 28]. Many arguments about the nutritional necessity of meat for humans focus on the concentration of protein and essential amino acids. The nine amino acids essential in human diets are conserved across the mammalian class, although the necessary daily intake values may vary. Data describing necessary daily protein and amino acid intake for great apes are limited, but several studies find that nonhuman primates require substantially more protein per kilogram body weight than do humans [29, 30, 31, 32, 33, 34, 35, 36, 37]. We know of only one study to experimentally assess chimpanzee protein requirements, and the authors were unable to ascertain the minimun daily protein requirement [29]. In zoos, chimpanzees typically eat monkey chow comprised of 15 to 25 percent protein [38], and consume between 2000 and 3000kcal per day [39]. This equates to the consumption of 75 to 187g protein per day, or between 1.3 and 4.7g per kilogram body weight. In the wild, they are able to meet protein requirements through a largely frugivorous diet. Humans require between 0.5g and 0.75g protein per kilogram body weight, and cannot safely eat more than about 1g/kg body weight. This is well below estimated chimpanzee protein intake. Of daily protein, adult humans require approximately 11 percent in the form of essential amino acids [40], and children require 35 percent [41].

Perhaps the most striking difference between traditional human and wild chimpanzee diets is the amount of dietary fiber. Chimpanzees eat up to 300 grams of fiber a day [42], while human foragers typically eat 70 to 90 grams per day. Analysis of human coprolites suggests intake of up to 130g per day in early Holocene populations [20]. Because some of the protein in plant foods is contained within the fibrous portion, the digestibility of plant protein is typically lower than that of animal foods, ranging from 84 to 97 percent [43].

This means that chimpanzees eating high fiber plant foods must eat larger quantities in order to meet daily protein requirements than humans eating a low fiber diet. This likely explains high chimpanzee protein consumption relative to humans. Due to the relatively lower protein bioavailability in plants [19, 44, 45], conventional wisdom holds that humans can more efficiently meet physiological requirements for protein through consumption of animal foods. It is possible for humans to consume adequate concentrations essential amino acids through consumption of a variety of plant foods in combination [46], but the idea that humans cannot ferment plant fiber is pervasive in the literature.

Compared with gorillas and chimpanzees, the human gut is smaller than expected for an ape of our body size. Proportionally, the human colon is short and the small intestine long compared with the great apes. However, humans and chimpanzees have a similar mean transit time for digestion, with high fiber foods passing more quickly [18]. This allows both species to eat large volumes of low-quality foods. A comparison of human and chimpanzee digestions suggests a similar capacity for fiber fermentation [43, 20]. Fiber fermentation occurs in the colon, where cellulolytic bacteria digest some portion of the fiber consumed [43]. It is estimated that humans today acquire upwards of 10 percent of daily energy from volatile fatty acids produced by fiber fermentation in the colon [47]. There is considerable individual variation in human ability to ferment fiber [48], and there is some evidence for enrichment in fiber digesting bacteria in fecal samples from populations with high fiber diets [49].

These observations suggest that plant foods are a viable source of protein for traditional human and prehuman populations. Ethnographic observation shows that San foragers obtain up to two thirds of their daily protein requirements from plant sources [50]. Additionally, consumption of raw animal protein may incur substantial digestive and masticatory costs [51, 45]. There is no firm evidence for widespread controlled use of fire before 400ka [52]. The net energy gained from raw animal foods might not be substantially higher than that from plant foods, despite higher nutrient bioavailability. We propose that the nutritional value and evolutionary significance of high protein animal foods are overstated in the literature.

Recently, the focus on meat in human evolution has shifted towards recognizing the importance of fatty acids from animal foods. The archaeological record points to hominin consumption of fatty animal foods like marrow [19] and brains [53]. Both marrow and organ meats are rich sources of essential fatty acids [21]. Fatty foods were likely crucial in offsetting the potential risks of high protein consumption for early hominins in the dry season

[54, 55, 56]. Further, some researchers argue that expanding brain sizes in the hominin lineage required increased consumption of essential fatty acids. Relative to nonhuman primates, humans require more essential omega-3 and omega-6 fatty acids [37]. Specifically, larger brain size in the genus *Homo* indicates increased requirements for acid (AA) and decosahexanoic acid (DHA), fatty acids involved in brain development. DHA and AA are most common in aquatic animals [57] and organ meats/brains from terrestrial herbivores [21, 58].

DHA and AA are considered essential in Western diets due to limited synthesis in the body. However, recent studies [59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 56] show that humans can synthesize DHA and AA from alpha-linoleic acid and linoleic acid, respectively. These fatty acids are common in plant foods. Linoleic acids is the primary fatty acid in seed oils, and alpha-linoleic acid is at highest concentrations in seeds, nuts, and legumes, but also occurs in significant quantities in leafy greens and fruits [69]. Interestingly, infants are able to synthesize DHA and AA more efficiently than adults [62], and women synthesize DHA and AA more efficiently than men [64, 65]. Further, DHA synthesis is more efficient in humans who eat a diet depleted in DHA than in those who consume sufficient dietary DHA [59, 60, 61, 63, 66, 67, 68]. Fatty plant foods such as mongongo nuts and baobab seeds play an important role in the diets of !Kung [70] and Hadza [71], respectively, and we cannot discount the potential importance of high fat plant foods in hominin diets.

The nutritional importance of protein and fat from animal sources is more equivocal than paleoanthropologists admit. In this paper, we discuss the nutritional contributions of wild plant foods to human diets, and present data on the macronutrient and amino acid composition of plants targeted by Twe forager-horticulturalists in Northwest Namibia. Data on the nutritional composition of wild plants targeted by human foragers is available in the literature (e.g. [72, 73, 74, 71, 75, 76, 77]), but this is one of the first anthropological studies to provide estimates of metabolizable energy content in order to control for reduced nutrient bioavailability in plant foods. We also assess protein quality in plant foods through amino acid analysis, and ask whether the Twe are able to meet daily requirements for protein and essential amino acids with a diet focused on wild plant foods. We use our observations of Twe plant consumption to draw inferences about the relative contributions of plant versus animal foods in hominin diets.

2.1.1 The Twe

The Twe are particularly appropriate for this study because their diet today is largely plant based. The Twe are forager-horticulturalists living in the Kunene Region of Namibia

and Southwest Angola. In this study, we focus on Twe living along a seasonal tributary of the Kunene River in the Otjitanga Valley, an inter-montane valley in the Zebra Mountains (Figure 2.1). The Zebra Mountain range is located at -17.133 S, 13.45 E, at 779m above sea level. The mountains are made of anthracite, with strips of acacia shrubs and other trees which lend a striped, zebra-like appearance. The Otjitanga Valley is seasonally dry and the area receives approximately 150-250mm rainfall annually [78]. Most of the valley is mopane (*Colophospermum mopane*) woodland with approximately 2000 trees and shrubs per hectare [78], although the riparian corridor along the Otjitanga riverbed supports large trees, including fig (*Ficus sycomorus*), ebony-wood (*Diospyros mespiliformis*), and clusters of Makalani palm (*Hyphaene petersiana*).

References to Twe in the historical literature indicate that the Twe lifestyle in Namibia has changed dramatically over the last century. Traditionally, the Twe were large game hunters and iron workers [79, 80]. Twe villages were located in the mountains in close proximity to most foraged plant foods, but hunters tracked large game in the valleys. Some Twe still live in the mountains, but today, many Twe spend a great deal of time at semi-permanent villages in the valleys. Since the Namibian war for independence (1966-1990), most Twe in Namibia have adopted elements of the Himba pastoral lifestyle, including dress and maize gardening, although very few Twe own livestock. Many Twe men are still involved with iron working, and trade iron beads for goats, sheep, or other goods.

Wildlife populations in the Kunene are depleted due to pastoralism and extended warfare, and large game specialization is no longer a feasible subsistence strategy. In fact, hunting game animals without a permit is illegal, and Twe do not have money to purchase permits. For this reason, we do not discuss hunting, but our observations suggest that hunted large game makes a minimal contribution to the diet. Twe men and women continue to forage for a variety of plant foods that grow in the mountains and along seasonal tributaries of the Kunene River. In Angola, many Twe live a more traditional hunter-gatherer lifestyle, and many of the participants in this study regularly travel between a number of locations in Angola and Namibia.

In 2007, following a severe drought, the Namibian government established three government camps in the Kunene region. Twe living at these camps receive a more-or-less monthly maize meal ration, small herds of goats, and are encouraged to plant gardens. The maize meal subsidies have become an important supplement to Twe subsistence work, to the point of becoming a staple. This is unsurprising given frequent drought that prevents successful gardening, as well as limited access to hunted meat. However, the maize subsidies

alone do not provide adequate calories, fat, or protein for active adults. The government goats cannot be used for meat, but during the rainy season are a source of sour milk. Most Twe do not own enough goats to produce sufficient sour milk to provision an entire family. Many Twe plant gardens when the rain allows, and occasionally hunt for small game like birds and rock hyrax. Gathered wild plant foods continue to comprise a large portion of the Twe diet.

This paper focuses on Twe who live at two settlements in the Otjitanga Valley, the Otjomuru Government Camp and Okau, a traditional settlement about 10km from Otjomuru (Figure 2.1). We also worked with Twe at households spread throughout the valley between these two settlements. The Twe share this valley with Himba pastoralists. There are at least two large Himba settlements in close proximity to the Twe camps, and there is regular interaction between the two groups. Most Twe are semimobile, and move seasonally to access different resources. Twe at Okau live a more traditional nomadic lifestyle than those at the Otjomuru government camp. Twe at both locations receive maize meal from the government, but in general, Twe at Okau are more involved in gardening than those at Otjomuru. Twe at both locations regularly forage for wild plant foods.

2.2 Methods

2.2.1 Interviews

Leonard conducted interviews with 88 participants with the help of a translator between July and October 2012, May 2013, and April and June 2014 to determine which wild plant foods are collected and consumed, which are consumed most frequently, and which cultivated and commercial foods are eaten most often.

2.2.2 Plant Collection and Identification

Leonard collected samples of wild edible plants available in the late rainy season/early dry season 2014 (April-June) with the help of Twe participants. One plant (*Ficus sycomorus*) was obtained from a Twe participant who had dried some of the fruits during the rainy season. We also collected samples of dried ground maize and millet from Twe gardens, as well as the government supplied maize meal. All plants were collected and exported with permission from the Namibian Ministry of Environment and Tourism. After collection, plants were cleaned and weighed, then air-dried and stored with silica gel. Dried samples were weighed again. Dried samples were stored in paper envelopes until they were needed for nutritional analysis. All plants were identified by botanists at the Namibian National Botanical Research Institute.

2.2.3 Nutritional Analyses

We measured dry matter, crude ash, crude protein, crude fat, crude fiber, neutral detergent fiber, acid detergent fiber, lignin, and estimated nitrogen free extractives, nonstructural carbohydrate, and amino acid content according to standard methods. Appendix A provides a full description of the methodologies used. Gross energy was estimated using Atwater factors 4kcal/gram for carbohydrate and protein and 9kcal/gram for fat. Metabolizable energy was estimated according to the protocol in Conklin-Brittain et al. [81].

We assessed the overall amino acid content of the plant portion of the Twe diet by summing the total amino acid content of various potential dietary combinations. These combinations are based on what is available in combination seasonally, and quantities are based on likely daily intakes estimated from informal interviews and observations collected during camp scans.

2.3 Results

2.3.1 Twe Diet

The Twe eat a variety of wild plants of a daily basis. Plant availability is highly seasonal. Table 2.1 lists the plants most commonly included in the Twe diet, and includes a description of seasonality and dietary importance. The following analyses focus on plants available in the early dry season. The foraged foods most frequently targeted at this time fall into two categories, fruits and underground storage organs. *B. discolor* berries and *D. mespiliformis* fruits comprise the majority of foraged foods this time of year. However, tubers from *F. angustifolia* are also taken frequently, and children often collect *Lapeirousia sp.* and *Campthorrhiza sp.* grass corms. Tubers from the leguminous plant *T. esculentum* are also available this time of year, but the Twe do not collect them. Several participants stated that this was a staple food prior to the government provisioning program, but since the establishment of the program in 2007, *T. esculentum* has completely dropped from the diet.

Table 2.2 shows the macronutrient and caloric content of Twe plant foods. The metabolizable energy values of these foods are low relative to the gross energy content. This difference is largely due to the high NDF (neutral detergent fiber) content of most of these foods, which decreases protein digestibility. Many of these foods have a high protein content. Of the wild plant foods, *T. esculentum* tubers and *Campthorriza sp.* grass corms are particularly high in protein, as are *B. discolor* and *F. sycomorus* fruits. Many of the wild plants also have a moderate fat content, with the values from *B. dicolor* berries and

Campthorriza sp. corms approaching those of *Z. mays* (maize) and *P. glaucum* (pearl millet).

Figure 2.2 shows the percent protein and fat in Twe wild plant foods in graphical form. Relative to protein, the fat content of these plants is quite low. The two grass corms, *Campthorriza sp.* and *Lapeirousia sp.*, have the highest percent fat, followed by B. discolor berries.

2.3.1.1 Amino Acid Content of Twe Foods

Appendix A. shows the entire amino acid spectrum for each plant.

Table 2.3 shows the essential amino acids content of Twe plant foods. The domesticates (*Z. mays* and *P. glaucum*) have a high concentration of many essential amino acids. Relative to the other wild plant foods, the grass corms (*Lapeirousia sp.* and *Campthorrhiza sp.*) have a high concentration of essential amino acids. In general, most of the wild plant foods are depleted in methionine relative to the domesticates.

Table 2.4 shows the dietary intake of essential amino acids, total protein, fat, fiber, and calories given different combinations of plant foods. Many of these dietary combinations approach the Word Health Organizations recommended daily intake for many of the essential amino acids. However, most of these diet combinations fall short of daily recommended values for methionine, isoleucine, and tryptophan, although maize-based diets come closer to recommended values for isoleucine. Even in a diet comprised only of wild plants, these combinations come close to the daily recommended protein intake for a 60 kilogram adult (45g protein per day, based on 0.75g protein/kg body weight) and the recommended minimum daily fat intake of 10 percent of total calories (or 20g for a 2000 calorie diet). All of the wild plant combinations fall far below the recommended daily fat and caloric intake, even when considering gross energy rather than metabolizable energy. However, in each of the wild plant food combinations, the fiber content is well below a daily maximum healthy value of approximately 100g/day. This suggests that the Twe could eat twice as much plant material. Further, because many of these foods are cooked, we suspect that the actual energy derived from these combinations falls closer to the gross energy. These estimates to not take into account other importance plant foods like seeds and nuts, which are high in both fat and calories, or honey, which is a very importance source of calories on a seasonal basis.

2.4 Discussion

Current hypotheses on the evolution of hominin diets maintain that the consumption of animal foods was a critical development in the evolution of the genus *Homo*. Animal meat is a calorically dense source of high-quality protein, fatty acids, vitamins, and minerals, and the potential nutritional contributions of animal foods to hominin diets have been thoroughly explored [25, 17, 82, 21, 20]. The focus on hominin meat consumption has detracted attention from the potential role of plant foods in hominin diets. Our work with the Twe suggests that humans living in semi-arid sub-Saharan African environments come close to meeting physiological requirements for protein and fat through a largely plant-based diet.

Table 2.2 shows the macronutrient content, gross energy, and metabolizable energy (ME) content of Twe plant foods. The metabolizable energy estimates account for lower protein bioavailability of plant foods due to NDF content, and assumes a moderate amount of fermentation in the colon. Although our discussion focuses on wild plant foods in the Twe diet, maize meal from the Namibian government comprises the bulk of the diet for most of our participants today. Maize is high in metabolizable energy, protein, and fat, and as such is an important source of nutrition for the Twe. Based on informal interviews and qualitative observations, we estimate that most adult Twe eat approximately 200 grams of maize meal each day. Gardened pearl millet (*P. glaucum*) is also an important dietary constituent when gardening is feasible. Like maize, millet is a good source of protein and fat. Both grains contain high concentrations of many essential amino acids, but a diet comprised solely of maize or maize and millet does not provide enough complete protein, and is likely depleted in many essential vitamins and minerals. Further, our estimate of 200g maize meal/day does not provide enough metabolizable energy to sustain an active adult.

Today, wild plant foods are an important supplement to the maize based diet. Not only do they provide an additional source of calories, but they also increase protein and fat consumption. In addition, many wild plant foods are rich in vitamins and minerals [83, 84]. Table 2.1 details the dietary importance of wild plants. Prior to maize dependence, the Twe exploited a greater variety of plants and plant types. Due to the depletion of game populations, hunting has not been common for at least twenty years and the Twe were dependent on wild plants for most of their dietary needs until 2007. Conventional wisdom holds that this is not possible given the constraints of the human digestive system, but a large body of research suggests that humans can ferment fiber and digest at least some of

the nutrients contained within. Further, comparisons of human and chimpanzee digestive kinetics suggest a comparable ability to ferment fiber despite differences in gut anatomy.

The protein content of wild Twe plant foods ranges from 2 to 12 percent (Figure 2.2). *D. mespiliformis* fruits, which are an important dietary constituent in the early dry season, have the lowest protein content, even relative to the other fruits. *B. discolor* berries and *F. sycomorus* fruits are 5.4 and 6 percent protein, respectively. With the exception of *Lapeirousia sp.* grass corms, all of the underground storage organs exceed 6% protein in dry matter. *T. esculentum* tubers are particularly high in both protein and fiber; the Twe eat these only after extensive roasting. High fiber *B. discolor* berries and *F. sycomorus* fruits are also sometimes boiled prior to consumption. We suspect that cooking increases the metabolizable energy portion of these foods by breaking down fiber [85]. Hypothetical dietary combinations listed in Table 2.4 show that a diet comprised wholly of wild plant foods can provide sufficient dietary protein.

Despite the high protein content of Twe wild plant foods, analysis of amino acids suggests that some animal foods might be necessary in the early dry season Twe diet. In wholly plant-derived diet, meeting the necessary intake of essential amino acids (aa's) may be challenging. Humans cannot synthesize essential aas, and must procure them from dietary sources. Essential aas are important for protein synthesis, and amino acid deficiencies can affect brain and immune function, absorption in the gut, and kidney function. Even when maize is included, most of the dietary combinations listed in Table 2.4 fall below the World Health Organization daily recommended intake for methionine, isoleucine, lysine, and tryptophan for adult humans [41]. These essential amino acids are plentiful in red meat, poultry, fish, and eggs.

Campthorrhiza sp. and *Lapeirousia sp.* grass corms have high concentrations of essential aas relative to other foraged foods, but they are rarely included in adult diets today. Their inclusion in a diet comprised mostly of *T. esculentum* tubers would improve the protein quality dramatically. Both species are regularly collected and consumed by Twe children. They are typically eaten raw, although brief roasting (5 minutes) over hot coals makes it easier to remove the outer covering (tunic). These corms are easily collected by children as young as 5 years old, and our observation suggests that a child can collect as much as 50g dry weight/hour. Both corms are also low in fiber relative to other wild plant foods, suggesting higher protein/amino acid bioavailability. Consumption of grass corms may help Twe children meet their elevated essential amino acid requirements.

Most of the wild Twe plant foods we sampled contain moderate amounts of fat. *B.*

discolor berries are notably high in fat compared with other wild plant foods, and are a preferred food in the Twe diet. *Campthorriza sp.* grass corms and *F. sycomorus* fruits are also good sources of fat. Prior to the government maize provisioning program, the Twe were heavily reliant on porridge made from grass seeds collected by harvester ants, and also regularly consumed baobab seeds. Both grass seeds and baobab seeds are high in fat [72, 71]. Today, the Twe obtain much of their required fat from regular consumption of maize meal (*Z. mays*), as well as gardened millet (*P. glaucum*). Essential omega-3 and omega-6 fatty acids are common in plant sources.

Our data suggest that the most substantial drawback of a completely plant-based diet is the ability to consume sufficient calories. The wild plant combinations listed in Table 2.4 are very low in calories, especially when considering metabolizable energy estimates. However, these diet combinations are also low in fiber, and the Twe could likely consume more than two times as much plant material. None of these dietary combinations include calorie-rich, high fat plant foods like nuts and seeds, because we were unable to obtain these for measurement. Prior to maize dependence, both baobab seeds and grass seeds were important dietary constituents. In a diet based on wild plants, high fat plant parts are likely crucial in meeting total energy requirements. Honey is also an importance source of readily digestible energy for the Twe. Honey is high in glucose and complex sugars, and is typically harvested in large quantities.

2.4.1 Implications for Human Evolution

Our data show that humans can meet most of our nutritional requirements through the consumption of wild plants. The Twe consume a combination of plant types with different qualities. Dry adapted underground storage organs in the Twe diet are high in protein, as are several wild fruits. Underground storage organs and fruits included in the Twe diet also contain moderate amounts of fat. High fat seeds were formerly dietary staples. Adequate intake of both fat and complete protein is necessary for proper physiological functioning, and so we suggest that certain plant types are more attractive in a meat-limited diet than others. These are high fat nuts and seeds and amino acid-rich grass corms.

Nuts and seeds are an important source of protein and fat for many extant foragers [70, 71]. In Africa, fatty plant foods include mongongo nuts, baobab seeds, palm nuts, and grass seeds. Many of these foods are widespread throughout the continent, and occur in a variety of habitat types. Plant oils are rich in essential omega-3 and omega-6 fatty acids, and it is possible that early hominins could process these resources with simple stone tools. Australopith cranio-dental morphology supports an adaptation to crushing large seeds and

nuts [86], and later hominins are associated with stone tools that could have been used for crushing. The high fat content of grass seeds may explain why they appear so early in the dental calculus record [87, 88].

High protein grass corms may have been similarly crucial to hominins trying to meet daily protein requirements. Like other USO types, grass corms provide a predictable, low variance resource, but caloric return rates may be much lower than for larger USOs. However, grass corms require very little effort to extract, and are easily collected with small digging sticks. The grass corms favored by Twe children are much lower in fiber than other underground storage organs in our sample, and do not require preconsumptive processing. These grass corms are also enriched in essential amino acids relative to other plant foods, and dramatically improve the balance of essential amino acids in the Twe diet. Edible corms from the genus *Lapeirousia* are widespread throughout Southern Africa [89], and may offer similar nutritional benefits to the corms sampled in this study.

2.4.1.1 How Much Animal Food Is Necessary?

Even in an environment where high protein plant foods are available, consumption of a small amount of animal food may be necessary to meet requirements for amino acids and calories. One hundred grams of steak supplies a sufficient quantity of essential aas to meet an adults daily requirements, and given that the Twe come close to meeting daily requirements through consumption of wild plants alone, we suspect that a much smaller portion of animal protein is sufficient. Most hypotheses on the importance of animal foods in human evolution focus on meat from large herbivores (e.g.[90]), and we cannot ignore ample evidence for the consumption of large ungulates early in the hominin lineage. However, our data indicate that the importance of hunted large game was not *nutritional*. Easily collected small game and insects provide the same nutritional benefits, and may have been important sources of calories, fat, and protein in hominin diets.

Ethnographic work supports the probable importance of small game in a variety of habitats. Small mammals, lizards, fish, shellfish, and insects are important food sources for many foraging groups [91, 92, 93, 94, 95, 96, 97]. Small mammals may have been particularly attractive due to high abundance and predictable distribution [97, 98]. Among extant foragers, small game acquisition is not a patently male activity like large game hunting. Women regularly target small game in many foraging groups, and children are able to collect some small animals as well. Among the Twe, women regularly fish and trap birds, even while pregnant and breastfeeding. Older children are also very active in hunting rodents. Men target small mammals including duiker, dik-dik, porcupine, and anteater.

Insects also make important dietary contributions among the Twe, and in several other traditional cultures [94, 95, 96], including the Hadza [93]. The Twe target mopane worms, which are seasonally very abundant, and high in both crude protein and fat [99]. Insects vary widely in nutrient composition [94, 95, 96], but are typically high in fat and protein, and provide similar nutritional benefits to animal meat. Preconsumption processing such as removal of inedible parts or cooking can alter the macronutrient content and improve digestibility [96]. Archaeologically, insectivory may be difficult to identify, although tools associated with *P. robustus* from Swartkrans, Sterkfontein, and Drimolen may have been used to dig termite mounds [95].

2.5 Conclusions

The Twe provide an instructive example of a plant-based dietary strategy that was also feasible for Plio-Pleistocene hominins. Anthropologists have long used extant foraging groups as a referent for hominin dietary behavior, but have focused on one or two groups at the expense of understanding foraging behavior in a broader range of habitats. The Twe live in a habitat type with different vegetation and resource availability than the Kalahri regions inhabited by the !Kung, that is also significantly dryer than the region along Lake Eyasi inhabited by the Hadza [100]. Hominins were distributed throughout the African continent, and Plio-Pleistocene climate reconstruction indicates that they lived in a variety of habitats with different degrees of aridity [101]. The extent to which hominins relied upon plant versus animal foods would have varied considerably with resource availability in any given habitat. This makes it extremely difficult to assume that any single dietary strategy "made us human." Cataloguing and understanding the variability of dietary strategies in extant foragers can help us draw inferences about past dietary behavior.

However, understanding contemporary human foraging choices can only take us so far. Nutritional science is perpetually changing, and our understanding of human nutritional requirements and digestive capabilities is far from complete. The one constant in our understanding of human nutrition is the adaptability of human populations to a broad range of dietary strategies. We are only beginning to understand the ways in which humans adapt to different diets through commensal bacterial communities. Research on nutritional requirements of great apes is far behind that of humans, and better documentation of how our physiological needs compare to those of our closest living relatives will highlight those aspects of human diet which are derived and require an evolutionary explanation.

Figure 2.1: Locations in Namibia mentioned in the text
Twe settlements Otjomuru (red) and Okau (yellow)

Table 2.1: Foraged plant foods in the Twe diet

Plant Name	Plant Part	Season	Preparation	Dietary Importance*
Berchemia discolor	berries	rainy season, early dry season	raw, often dried	1
Camptorrhiza sp.	corms	early dry season	briefly roasted to remove tunic, then eaten raw	2
otjihakariwa	tuber	early dry season, dry season	raw	2
Coccinea sessilifolia	tuber/corm	rainy season	roasted	3
Cyperus fulgens	tuber/corm	rainy season	roasted	3
Diospyros mespiliformis	fruit	early dry season	raw, often dried	1
Ficus sycomorus [fig]	fruits	rainy season	raw or dried and ground, cooked as porridge	1
Fockea angustifolia	tuber	early dry season, dry season	raw	1
Grewia bicolor	berries	early dry season, dry season	raw	1
Grewia flava	berries	early dry season, dry season	raw	1
Grewia flavescens	berries	early dry season, dry season	raw, soaked	1
Grewia schinzii	berries	early dry season, dry season	raw	1
Grewia tenax	berries	early dry season, dry season	raw	1
Grewia villosa	berries	early dry season, dry season	raw	1
Gynandropsis gynandra	leaves	rainy season	boiled	1
Hyphaene petersiana [Makalani palm]	nut	dry season	raw or ground and cooked as porridge	2
Lapeirousia sp. [ozonduvi]	corms	early dry season	briefly roasted	1
Salvadora persica	berries	dry season	berries boiled	1
Sclerocarya birrea [marula]	fruits	rainy season	raw	2
Tylosema esculentum	bean and rhizome	tuber, year round, bean rainy season	bean preparation unknown, tuber roasted	3
Vangueria ingausta	fruit	rainy season	raw	2
Ximenia americana	fruit	rainy season	raw	1
Ziziphys mucronataa	berries	rainy season, early dry season	raw	2
otjihakariwa	tuber	early dry season, dry season	raw	2

*1=frequently consumed, 2=infrequently consumed, 3=formerly important food, no longer consumed.

The table details plant part(s) consumed and season(s) of consumption, as well as methods of preparation. The final column lists the dietary importance/frequency of consumption, which is estimated through qualitative observations and interviews.

Table 2.2: Macronutrient content of foraged and gardened Twe plant foods, dry plant material

taxon	% moisture	%protein	%fat	% crude fiber	%NDF/textsuperscript1	%carbohydrate[2]	Gross Energy[3]) (kcal/100g)	Metabolizable Energy[4] (kcal/100g)
Berchemia discolor		6	3	5	14	72	432	350
Diospyros mespiliformis		2	2	21	35	44	440	274
Hyphaene petersiana		4	2	36	42	18	444	210
Fockea angustifolia		7	3	9	18	58	385	295
Lapeirousia sp.		6	3	6	16	64	411	328
Camptorrhiza sp.		7	4	2	1	84	429	390
Ficus sycomorus		6	2	16	37	44	447	298
Tylosema esculentum		13	2	19	33	32	438	265
Pennisetum glaucum		13	4	8	4	57	470	360
Zea mays (Twe Gardens)		12	4	7	4	60	452	350
Zea mays (Government)		9	4	4	3	74	454	384

[1]Neutral detergent fiber, includes plant structural components which are not readily digestible, indigestible lignin, and low fermenting polysaccharides like cellulose and hemicellulose. NDF is the sum of plant structural components, and is reported as a percent of the total fiber content

[2]nonstructural carbohydrates were estimated using measured nitrogen free extrative values

[3]gross energy was calculated using 4kcal/g for carbohydrate and protein and 9kcal/g for fat

[4]metabolizable energy is an estimate of the digistible portion of the gross energy content.

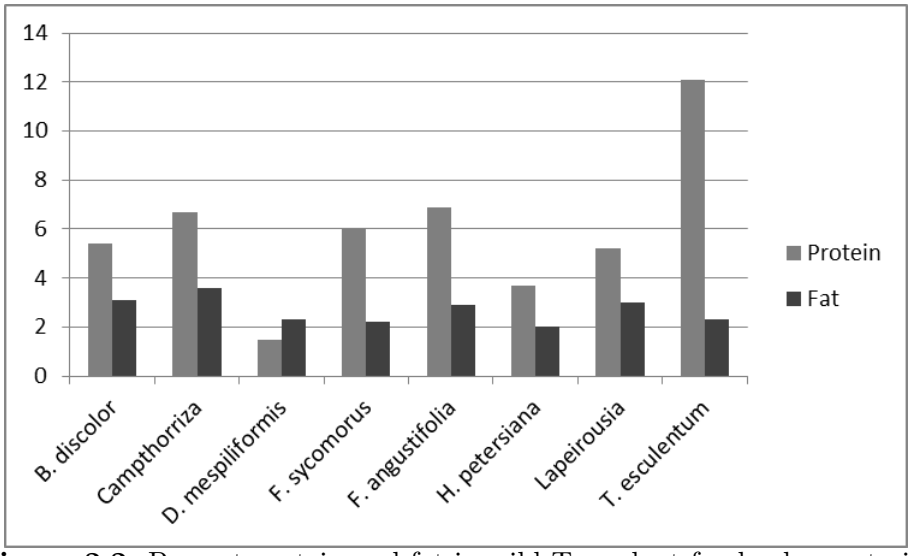

Figure 2.2: Percent protein and fat in wild Twe plant foods, dry material

Table 2.3: Essential amino acid content of Twe plant foods

	Methionine	Threonine	Valine	Isoleucine	Leucine	Phenylalanine	Histidine	Lysine	Trpytophan
					mg/100g				
Zea mays (Government)	271	225	430	227	1168	399	216	229	9
Zea mays (Twe Gardens)	278	446	641	346	1438	545	268	481	17
B. discolor	26	97	135	82	184	97	46	93	4
D. mespiliformis	24	88	111	67	142	78	51	137	7
Campthorrhiza sp.	169	321	419	237	480	387	173	222	20
F. sycomorus	80	277	387	248	507	297	140	292	13
F. angustifolia	31	126	133	87	138	84	72	151	0
H. petersiana	29	128	174	104	235	126	53	180	0
Lapeirousia sp.	53	196	224	125	307	126	118	216	2
T. esculentum	51	134	274	118	171	122	215	255	6

Essential amino acids cannot be synthesized in the human body, and must come from dietary sources. They are involved in brain, immune system, and kidney function, and a deficiency can have serious consequences for health. All values are shown in mg/100g.

Table 2.4: Essential amino acid content of possible Twe diet combinations

WHO recommended daily intake[1]	methionine 1050	threonine 1050	valine 12820	isoleucine 1400	leucine 2730	phenylalanine 1750	histidine 700	lysine 2100	tryptophan 280	dietary intake[2]	fiber	fat	protein	kcal[3]	metabolizable kcal
food combination															
200g maize meal, 100g *B. discolor*, 100g *F. angustifolia*	598	673	1129	624	2657	979	551	702	22		15	38	86	1093	856
200g government maize meal, 100g *B. discolor*, 100g *F. angustifolia*, 100g *D. mespiliformis*	622	761	1239	691	2798	1057	602	839	29		26	41	87	1251	916
200g government maize meal, 100g *B. discolor*, 100g *Camptorrhiza sp.*	761	965	1550	857	3183	1379	698	897	48		12	44	89	972	871
300g government maize meal, 100g *B. discolor*, 100g *F. angustifolia*	869	898	1559	851	3824	1378	767	932	31		18	42	125	1547	1240
300g government maize meal, 100g *B. discolor*, 100g *F. angustifolia*, 100g *D. mespiliformis*	893	986	1670	918	3966	1455	818	1069	38		38	58	126	1705	1298
300g government maize meal, 100g *B. discolor*, 100g *D. mespiliformis*, 100g *Camptorrhiza sp.*	1062	1307	2089	1155	4446	1842	991	1292	58		26	59	129	1584	1313
200g *T. esculentum*, 100g *B. discolor*, 100g *F. angustifolia*	159	491	817	406	663	425	548	753	16		32	12	26	595	259
200g *T. esculentum*, 100g *B. discolor*, 100g *F. angustifolia*, 100g *D. mespiliformis*	183	579	928	473	805	502	599	890	23		43	15	27	753	327
200g *T. esculentum*, 100g *B. discolor*, 100g *Camptorrhiza sp.*	351	900	1346	710	1284	889	771	1113	43		29	13	21	474	214
300g *T. esculentum*, 100g *B. discolor*, 100g *D. mespiliformis*	253	773	1315	654	1163	714	839	1224	40		47	15	32	814	347
300g *T. esculentum*, 100g *B. discolor*, 100g *D. mespiliformis*, 100g *Camptorrhiza sp.*	422	1094	1734	891	1643	1101	1011	1446	60		51	19	35	837	362
300g *T. esculentum*, 100g *B. discolor*, 100g *D. mespiliformis*, 100g *Camptorrhiza sp.*, 100g *Lapeirousia sp.*	475	1289	1857	1017	1950	1227	1129	1662	62		54	22	37	887	386

[1] World Health Organization recommended daily intake, mg/70kg body weight.
[2] grams per day, fresh plant material

The top row shows the World Health Organization recommended daily intake for each essential amino acid as a reference [41]. The food combinations in the leftmost column are grouped into those that contain maize (top) and those that contain only wild plant foods, with former staple food *T. esculentum* replacing maize (bottom). Each hypothetical diet is a possible combination of plant foods in the Twe diet based on availability in the early dry season. Some combinations vary only in food quantities, rather than species.

[3] Caloric values are provided for fresh plant material. See Appendix A for methods use to calculate calories in fresh plant material.

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CHAPTER 3

NUTRITIONAL VARIATION AND PROCUREMENT COSTS OF AFRICAN UNDERGROUND STORAGE ORGANS

3.1 Introduction

Increased brain and body size and reduced tooth and gut size in the genus *Homo* are widely viewed as adaptations to a change in diet, but opinions differ on precisely what that change entailed. Conventional wisdom favors a scenario wherein greater access to meat, through a combination of hunting and scavenging, was a critical selective pressure for morphological and behavioral changes in early *Homo*. Support comes from a recognition that the earliest appearance of the genus *Homo* coincides with increasingly seasonal, arid climates that reduced access to previously important plant foods and simultaneously stimulated growth in large animal biomass [1, 2]. A concurrent increase in archaeological evidence for the consumption of animal foods lends further validation [3, 4, 5, 6, 7]. This argument also appeals to the nutritional qualities of meat and marrow, which may have supported the increased metabolic demands of a larger brain and body [8, 9, 10, 11]. The meat argument links consumption of animal foods to uniquely human behavioral traits like central place foraging and sexual division of labor [12, 13, 14].

Critics observe that the relative importance of meat in the diet of early *Homo* cannot be determined from archaeological data alone. Even if we accept that meat consumption increased in early *Homo*, the archaeological evidence does not indicate the scale of that increase. Further, the archaeological evidence does not indicate central place foraging or sexual division of labor. There is some evidence for transport of certain skeletal elements [15, 16], but little support for the idea that these elements were transported to base camps where they were widely shared [17], cf. [16]).

In the conventional argument, meat is a critical dry season resource due to the limited availability of plant foods at this time. Critics observe that the nutritional value of meat is diminished in the dry season. Meat from large African ungulates is very lean, with carcass

fat content typically less than 4 percent for most of the year, but around 1 or 2 percent by the end of the dry season [18]. Many dry season plant foods are also high in protein, suggesting that hominins subsisting largely on animal foods would have faced the negative health effects of excess protein consumption [19, 18, 20, 21]. San and Hadza forager body mass decreases during the dry season when meat consumption is highest, despite adequate caloric intake [22, 23, 24, 17, 19]. Furthermore, ethnographic data from modern analogues indicate that access to meat through either hunting or scavenging is inconsistent at best [25, 26, 24]. Meat acquisition would have been even more irregular in the past given more limited acquisition technologies.

The alternative argument proposes that plant underground storage organs (USOs) were key in the development of uniquely human physiology and behavior. USOs are widely distributed in a range of habitats and comprise a valuable source of calories for many extant foraging groups [27, 28, 29, 30, 23, 31, 32, 33, 34, 35, 36]. A survey of USOs targeted by Hadza foragers suggests that density and biomass is quite high, and that foragers can return to the same patch repeatedly before exhausting the edible supply [36]. Increasing aridity and seasonality in Pleistocene Africa lead to the spread of USO-rich habitats [37]. The presence of USO-consuming fossil rodents at hominin localities indicates that USOs were also available to Pleistocene hominins [37, 38].

Proponents of the USO hypothesis argue that USOs offer several nutritional advantages over other resource types [39, 40, 33, 38]. For example, USOs store water and carbohydrate through dry periods when other resources may be scarce, and as such may have been a seasonally important resource in savanna environments [33]. The substitution of USOs for other plant food types may lead to an overall reduction of dietary fiber [39]. Changes in thoracic shape in the genus *Homo* suggest a reduction in gut size, which is typically linked to the consumption of nutrient dense foods or more intensive processing prior to consumption [41, 38]. Finally, the rate of caloric acquisition for at least some USO species is quite high. The few published data on USO return rates range between 900-4500 calories/hour [27, 28, 29, 31, 32, 34, 42, 35, 43, 36]. This large variation in return rates suggests that categorical statements concerning nutritional qualities of USOs are problematic.

Critics of the USO hypothesis argue that these are secondary resources at best (e.g. [44]). As proponents of USO consumption point out, USOs are often buried deep beneath the surface, and difficult to acquire [33, 38]. Bunn [8, 15] maintains that hominins lacked the technology and skill to access deep-growing USOs, but Coursey (1973) argues that even the earliest members of the hominin clade could likely access USOs with simple digging

sticks. There is some evidence for digging sticks in the Pleistocene [45], and ethnographic work shows that a digging stick can be manufactured with the simplest stone tools in a matter of minutes [36].

Critics also dispute the nutritional utility of USOs. Recent analyses of USOs targeted by Hadza foragers suggests that USOs are low in calories, protein, and fat [46], and that glucose bioavailability is low [47]. Others suggest that the digestibility of raw USOs may be inhibited by a high fiber content [8, 15]. Cooking may increase digestibility of energy in plant foods by breaking down fiber and carbohydrate [48, 49, 38]. There is some evidence for controlled fire use as early as one million years ago [50, 51, 52], but this is contested [53, 54, 55]. It is worth noting that USO nutritional composition is variable even within the same species [46].

In this paper, we explore the potential nutritional contribution of USOs to hominin diets in two ways. First, we review published nutritional data on wild USOs used by African foragers and ask whether or not USOs are a high-quality resource. We compare USO nutritional composition to that of commonly consumed wild fruits, and discuss nutrient bioavailability. Second, we ask whether the energy expended while digging makes a substantial difference in USO caloric acquisition rates. To do so, we use heart rate increases to estimate energy expenditure of Twe foragers while digging for two USO species. Our dataset suggests that energy expenditure while collecting USOs may be considerable.

3.1.1 The Twe

The Twe are forager-horticulturalists who live in the Kunene Region of Namibia and Southwest Angola. This region is mountainous with a hot, dry climate, and receives approximate 150-250mm rainfall annually [56]. Most of the rainfall occurs in the rainy season between December and March.

The earliest references to the Twe come from as early as the 16th century, but the group was relatively unstudied until the 1960s, at which time the Twe were known as large game hunters and iron workers [57]. In the 1960s and 1970s, hunting and gathering and trading iron beads and tools were the primary forms of subsistence. However, by the end of the Namibian War for Independence (1966-1990), many Twe adopted aspects of the Himba pastoralist lifestyle, including small-scale maize gardening and womens dress style. Twe men continue to make iron beads and arrows which they trade for livestock or commercial goods, but few Twe are able to amass herds of cattle, goats, or sheep. Large game hunting is no longer a reliable source of calories. Game populations have declined in response to overgrazing/pastoralism and overhunting during the war. Twe women continue to forage for

a variety of wild plant foods, and both men and women target birds and small mammals. In 2007, the Namibian Government began a provisioning program for Twe living at government camps with more-or-less monthly shipments of maize meal. Many Twe establish home-bases at or near these government camps in order to receive maize rations, but divide their time between other villages in the region.

The data reported in this study were collected at a semipermanent Twe settlement called Okau, which is located 10km from a large government camp (Figure 2.1). Okau is a collection of compounds spread 1 to 3km apart on either side of a seasonal tributary of the Kunene River in the Otjitanga Valley. The Otjitanga valley is a narrow inter-montane valley in the Zebra Mountains. The riparian corridor supports large fruiting trees and clusters of Makalani palm (*Hyphaene petersiana*), which is used as a food source (palm nuts) and for alcohol. The rest of the Valley is covered with mopane (*Colophospermum mopane*) woodland. The surrounding mountains are rugged, and covered with anthracite boulders. Small patches of fruit trees and tubers occur on the hillsides, and large fruit patches are found on mountain top valleys.

Okau is located only 5km from the nearest Himba settlement. The Himba are pastoralists with large herds of cattle, but they do not graze on the land directly surrounding Okau. The two groups have regular contact and more or less cordial relations, but the Twe are considered socially inferior because of their relative poverty and recent hunter-gatherer background.

3.1.1.1 Twe Foraging

Today, the Twe regularly forage for at least six USO species (*Fockea angustifolia*, otjihakariwa (Herero name), *Lapeirousia sp.*, *Camptorrhiza sp.*, otjimaka (Herero name), and ozozeu (Herero name)). A seventh, *Tylosema esculentum*, was formerly a staple but has dropped out of the diet since the advent of the government maize-provisioning program in 2007. Several wild fruits are regularly included in the diet. We discuss the two most common here, as a counterpoint to USO foraging. Foraging for USOs is typically an independent activity, and most often performed by women. Many informants state that there is a stigma attached to digging for your food like an anteater. The most desired foods are meat, sour milk, maize, and honey, likely due to the influence of pastoralists, and our impression is that Twe try to hide their USO consumption from others. In addition, many informants say that they only eat USOs when there is little else available. In this paper, we focus on *F. angustifolia* and *T. esculentum* because *F. angustifolia* is currently the most frequently targeted USO and *T. esculentum* was formerly a major dietary constituent.

T. esculentum is a leguminous plant that grows in small clearings throughout the valley. It produces a groundcover vine with edible beans and a large tuber. These tubers grow deep in order to access groundwater, and Twe may dig more than a meter below the surface to acquire them. The tubers are thick and over a meter in length, and can weigh upwards of 200kg (Bergstrom and Skarpe, 1981), although Twe typically target younger, smaller tubers weighing in the tens of kilograms. The soil is hard and rocky, and digging these tubers requires significant physical effort, even with iron rods or hoes. Men are just as likely as women to collect *T. esculentum* tubers. Nutritionally, the tuber is a very good source of protein and contains a moderate concentration of most of the essential amino acids (see Chapter 2). However, it is also very fibrous. The Twe roast these tubers for an hour or more prior to consumption in order to break down the fiber, making the tuber more digestible and easier to chew.

F. angustifolia grows in rocky soil on the mountains (observation by CL; [58]). This tuber is available year round, but may be difficult to find in the dry season because the vine loses its leaves. The tubers are 90% water, but are nonetheless a reasonably good source of carbohydrate and protein (see Chapter 2). These tubers are not buried deep, but are often found beneath large rocks which must be moved in order to access the tuber. Some rocks are heavy enough to necessitate levers. The tuber itself is extracted using a simple wooden digging stick.

Twe also forage for several wild fruits. Unlike USO foraging, fruit foraging is a group activity and women will leave camp together in the morning with their children and spend several hours or days collecting the targeted fruits. In the early dry season, the most commonly collected fruits are *Berchemia discolor* berries and *Diospyros mespiliformis* fruits. *B. discolor* berries are found in large patches on mountain top valleys, and collecting trips usually last for several days. The closest patch to Okau is a half-day walk, approximately 5km through the valley and another 5km or more into the mountains. These berries are picked from the tree or from the ground beneath trees. Women collect as many as they and their children can carry, and dry them in the sun. They eat these berries throughout the dry season and sometimes sell them in town. *D. mespiliformis* fruits are found closer to camp along the dry riverbed, although people will travel quite far to find unused patches. The fruits are available for only a matter of days once ripened because they are a favorite of many birds and vervet monkeys. These fruits are also collected in huge quantities and dried for consumption throughout the dry season, or until they run out.

3.2 Methods

This paper uses data on wild USO and fruit nutrition and caloric acquisition rates from previously published work, but also presents new data on nutritional content, foraging times/quantities, and collection costs of USOs and fruits targeted by Twe at Otjomuru and Okau. Here we detail methods for data collection in the field as well as the assessment of starch content of Twe USOs and fruits.

CL collected foraging data and plant samples between April and June 2014 (early dry season). CL accompanied Twe informants on foraging trips and recorded the time spent searching for the resource, the time spent collecting, and the time spent processing a resource after collection. During fruit foraging trips, we noted the time spent eating in the patch and subtracted that from the total foraging time, so that the time recorded only reflects the time spent picking the amount weighed at the end of the trip. This protocol is problematic in that the berries that a forager eats while picking contribute to the portion of the total return that is not shared. However, on-site consumption is minimal on these trips, and weighing each piece of fruit prior to consumption or counting the total number of fruits consumed was not feasible. Hawkes et al. [22] control for eating during berry picking by calculating the collection rate during an exercise in which foragers agreed not to eat while picking. We suspect that Twe consumption is lower than recorded consumption rates for the Hadza, because Twe women converse rapidly while berry picking. We weighed tubers and fruits after collection, and later determined the weight of the edible portion. Weights were obtained using a digital scientific scale and rounded to the nearest gram. Return rates are reported as an average of calories acquired per hour for all foragers collecting the specific plant. We report return rates in terms of both total energy content and metabolizable energy content of fresh plant material (see below). Breakdown by individual collector would result in samples too small to have much analytic utility. Return rates are calculated as follows.

$$\frac{\text{Total Calories}}{\text{Collection Time} + \text{Processing Time}} \tag{3.1}$$

Methods for determination of caloric content in Twe plant foods are described in a previous paper (see Chapter 2). We followed standard procedures for determination of protein, fat, nonstructural carbohydrates, total fiber, acid detergent fiber, neutral detergent fiber, lignin, moisture, gross energy content, and metabolizable energy content. Metabolizable energy refers only to the digestible portion of the gross energy content, estimated based on the assumption that humans ferment fiber to a limited degree [59, 60, 61, 11]. Fresh weight caloric content was extrapolated using the following equation [62]:

$$\text{Energy content of a fresh food} = (\text{ME}/100g\text{OM})x(g\text{OM/g of the fresh food}) \quad (3.2)$$

In the current study, we determined starch content of Twe USOs and fruits. Starch content of dried plant material was determined using the Megazyme Total Starch Assay Kit. Briefly, the kit uses the amyloglucosidase/ α -amylase method, in which starch is completely solubilized during incubation in the presence of thermostable -amylase. Absorbance was measured in a Thermo Scientific Multiskan FC microplate reader.

We also collected heart rate data during foraging trips in order to estimate energy expenditure. We asked participants to wear heart rate monitors and recorded average heart rate while searching and average heart rate while collecting. We use heart rate increase from search to collecting as a proxy for increased energy expenditure while collecting. Although the link between heart rate and energy expenditure is not perfect, there is a linear relationship between heart rate and oxygen consumption between 90 and 150 beats per minute heart rate. We used a predictive model presented in Keytel et al. (2005)[63] to estimate energy expenditure from heart rate without the need for individual calibration or measurement of V02max, or maximum possible oxygen consumption during exertion. The equation is:

$$\begin{aligned}&\text{Energy Expenditure} = \\ &\text{gender}(-55.0969 + 0.6309\text{heart rate} + 0.1988\text{weight} + 0.2017\text{age}) + \\ &(1 - \text{gender})(-20.4022 + 0.4472\text{heart } 0.1263\text{weight} + 0.074\text{age})\end{aligned} \quad (3.3)$$

where gender = 1 for males and 0 for females. We estimated body weight of participants based on population averages (Layne Vashro personal communication on June 28, 2015). Age was determined in interviews. We are aware that these estimates are not perfect, but they nonetheless illustrate the potential increase in energy expenditure during different activities.

3.3 Results

3.3.1 Nutritional Qualities of African Underground Storage Organs

A review of data on edible USOs exploited by foragers in Southern Africa, Tanzania, Cameroon, and the Central African Republic shows that nutritional composition is highly variable. Table 3.1 shows moisture content, protein, fat, nonstructural carbohydrate content, total carbohydrate content, total fiber content, neutral detergent fiber content, and calorie content of several USOs. Information for each category was not available for all

USOs in the table. Nonstructural carbohydrate differs from total carbohydrate, which also includes structural carbohydrates like dietary fiber. Nonstructural carbohydrates include free glucose, starch, and other sugars. Total fiber includes acid detergent fiber, neutral detergent fiber, and lignin. Neutral detergent fiber (NDF) is the insoluble portion of the total dietary fiber. It is important to distinguish between total fiber and NDF, because NDF has a greater impact on the overall digestibility of plant foods. NDF is insoluble in the human intestine, and the energy contained within the NDF portion of a food is less accessible to human consumers than soluble portion of the total fiber content.

In general, most USOs in Table 3.1 are high in carbohydrate and low in fiber. Protein content ranges from negligible to very high, and fat content is consistently low, with only a few exceptions. Gross fiber content is generally lower than other edible plant food types (Table 3.2). Unfortunately, insoluble fiber (neutral detergent fiber/NDF) content was not published for most species. NDF content for the Namibian USOs from our analysis tends to be lower than for wild fruits, but NDF content in Hadza USOs is high.

Table 3.2 shows the nutrient content of fruits targeted by Twe and Hadza foragers for comparison with USOs. The fruits are comparable in caloric content relative to the USOs listed in Table 3.1, and much higher in fiber. NDF values were not available for most of these fruits, but the high values reported for the three fruits from Namibia contribute to the low metabolizable energy values for those fruits relative to the gross energy content. Fat and protein content in these fruits are variable, but fat content is consistently higher than that of USOs.

3.3.2 Return Rates of Twe Underground Storage Organs and Fruits

Table 3.3 shows return rates for Twe USOs calculated in terms of both total energy content and metabolizable energy content gained per hour, which accounts for the estimated digestible portion of each food. In each case, the metabolizable energy return rate is less than half of the total caloric return rate. The USO return rates are variable, but at least one is comparable to return rates of two commonly eaten fruits. The return rate for *Lapeirousia sp.* grass corms is very low; the corms are very small, and are usually collected by children.

Table 3.4 shows heart rate for two men digging tubers and for one woman picking fruits. The equation used to calculate energy expenditure from heart rate takes sex into account, and in general, men expend slightly more energy than women per kilogram body weight at the same heart rate. In both cases, the increase in heart rate from digging USOs is substantially larger than that from picking berries. Similarly, the increase in estimated energy expenditure is much larger for digging tubers than from picking fruits.

Table 3.5 shows return rates of Twe USOs and fruits from Table 3.3 recalculated to account for energy expenditure. The small increase in heart rate associated with picking fruits has a minimal impact on return rates for fruits 3.5. However, the large increase in heart rate associated with digging tubers noticeably decreases the net caloric return rate of *T. esculentum* tubers. The net return rate for *F. angustifolia* is very high regardless of the high energy expenditure associated with digging. We also show the return rates of Twe food using metabolizable energy content rather than gross energy content to correct for the indigestible NDF portion of these foods. If we subtract energy expenditure from the metabolizable energy return rates, *T. esculentum* is much less attractive.

Table 3.6 shows estimates of net return rates of Hadza USOs based on Twe energy expenditure estimates. The caloric contents reported in Vincent, 1985 are likely overestimates because they include the fibrous quid, which is chewed but not ingested. However, the estimated net return rates are still illustrative of the effects of high energy expenditure during digging on return rates.

3.4 Discussion

3.4.1 Nutritional Quality of African Underground Storage Organs

Opinion on the nutritional quality of USOs is divided. Proponents of the USO hypothesis appeal to the nutritional attractiveness of USOs as a source of calories and carbohydrate (e.g. [33]), while critics maintain that USOs are low in calories relative to other foods [46] with low digestibility in their raw state [8, 47]. Our review of published data suggests that the nutritional qualities of African USOs vary widely (Table 3.1). Some are very low in calories, but many are comparable to wild fruits on a dry matter basis (see Table 3.2). The calorie and specific macronutrient content will be lower in fresh plant material due to moisture content. In fact, some USOs contain as much as 90 percent water, and may be a valuable source of water in dry environments. Fat content is consistently low in African USOs, suggesting that USOs are not a good source of essential fatty acids. In comparison, several of the wild fruits listed in Table (Table 3.2) contain relatively high amounts of fat. Protein content, on the other hand, is very high in some USOs. Wild fruits also contain moderate to high amounts of protein. In a previous paper, we show that some USOs from Namibia contain a high concentration of essential amino acids, and are an important source of high-quality protein among the Twe.

Bunn [8, 15] critiques the hypothesized importance of USOs by appealing to their high fiber content, while Conklin Brittain et al. [39]) suggest that USOs are an attractive resource

due to their low fiber content. Most of the USOs listed in Table 3.1 contain low proportion of gross fiber, especially in comparison with the fruits listed in Table 3.2. However, the gross fiber content is comprised of both soluble (digestible) and insoluble (indigestible) fiber. The indigestible fiber portion is called neutral detergent fiber (NDF). NDF values are not available for most species, but given the variability of NDF content in Twe tubers, we assume that NDF varies similarly across the species listed in Table 3.1. With the exception of *T. esculentum* tubers, the NDF values we report for Twe tubers are much lower than for other resource types, like fruits, leaves, inflorescences shown in Conklin Brittain et al., [39] or most of the fruits for which we have NDF data in Table 3.2. Interestingly, the Hadza tubers collected in Tanzania are consistently high in NDF, even in comparison with fruits.

To control for limited digestibility, we provide estimates for metabolizable energy when available (Tables 3.1 and 3.2). Foragers can increase fiber digestibility (and metabolizable energy) with preconsumption processing. In particular, cooking can increase digestibility by softening indigestible fiber, and dry heat may even initiate the conversion of insoluble fiber to soluble fiber [49]. Other benefits of cooking include increasing carbohydrate digestibility and denaturing toxins [64]. Experimental work suggests that brief cooking is not sufficient to greatly increase digestibility [47], but longer cooking times may have a greater effect [49]. Cooking may also decrease chewing costs and the costs of digestion [65]. Among both the Twe and the Hadza, roasting is common, but the Twe also consume some USOs raw. Conventional wisdom holds that humans have a limited ability to ferment fiber in comparison with our great ape relatives [41], but there is increasing evidence that fiber fermentation is more than negligible [59, 66]. In fact, some estimates suggest that humans meet upwards of 10 percent of their daily energy needs through fiber fermentation in the colon [60]. This suggests that the focus on USO fiber content is at least somewhat misguided.

Because USOs are used for plant energy storage, the nutritional composition can vary dramatically by season. Some USOs completely replace the subterranean organ each ear. These lose mass and energy stores throughout the growing season. Others grow for several years and amass the nutrients needed for flowering annually. These have the highest mass just before flowering and again before the reproductive period [67]. The Twe are able to find *F. angustifolia* tubers throughout the year, but prefer to eat them in the dry season when they are swollen with water. We do not have nutritional measurements of *F. angustifolia* in different seasons, but visual observation using light microscopy shows that the starch content is higher in the dry season than the rainy season (108 granules/mg versus 48 granules/mg). Hadza tubers are also available year round, but the rate of consumption

fluctuates seasonally. Vincent [36] reports that consumption is highest during the main rainy season and the late dry season, and Hawkes et al. [23] observe that consumption is lowest in March at the end of the rainy season. We do not know of any data that suggest this seasonal exploitation pattern corresponds with the highest nutritional utility of these USOs, but this merits future exploration.

Our review shows that USO nutritional composition and quality is highly variable, to the point that using the label USO as a resource type is misleading. Some USOs are rich in calories, carbohydrate, and protein. Most are low in gross fiber relative to other plant types. However, some USOs are very low in calories, especially when considering the metabolizable portion. A more systematic study of seasonal variation in USO nutrition would elucidate how human foraging strategies respond to seasonal variation in USO nutritional status.

3.4.2 Energy Expenditure and Return Rates

Proponents of the USO hypothesis suggest that caloric acquisition rates while foraging for USOs are sufficient to support a forager and one or more dependents (e.g. [68, 23, 33]). Among the Hadza, grandmothers are able to enhance their fitness through provisioning grandchildren with USOs, and a similar type of food sharing may have selected for increased longevity in *Homo erectus* [33]. Published hourly return rates for USOs range from 900 to 4500 calories per hour, and our data from the Twe fit within this range (Table 3.3). Critics imply that these return rates may overestimate the actual caloric value of USOs given low digestibility of many USOs. Marlowe and Berbesque [44] suggest that body mass of Hadza foragers is lowest at camps with the highest USO consumption, and question the nutritional value of USOs. However, their analysis also shows that both body mass index and percent body fat are lowest in the wet season, when a higher variety of fruits and honey are available. Other studies find that forager body mass decreases in response to increased consumption of lean meat [69], rather than USOs. Our analysis/observation of Twe USOs shows that return rates of some USOs remain high even when considering only the metabolizable energy content (Table 3.5).

While accounting for metabolizable energy does not contradict the idea that USOs are a high return resource, the high energetic costs of USO acquisition may further decrease caloric acquisition rates. Our dataset for heart rate is small, but it illustrates an important point. Among the Twe, the increase in heart rate for digging for tubers is much higher than the increase in heart rate for picking fruits. Using heart rate increase, we estimate that Twe expend up to 700 calories per hour digging, compared with only 120 calories per hour picking fruit (Table 3.4). This corresponds to an increase over search costs (walking) of up

to 400 calories per hour over for digging, but only 66 calories per hour for picking (Table 3.5). These data indicate that USO return rates may be inflated if they fail to take energy expenditure into account. In some cases, caloric return rates are so high that accounting for energy expenditure makes little difference (eg. *F. angustifolia*). In others, subtracting the energetic costs of digging from the total calories gained may change the desirability relative to other resource types. Cooking may further decrease USO return rates.

The profitability for *T. esculentum*, for example, drops from 933kcal/hour to only 513kcal/hour, making this the least desirable Twe plant resource for which we have data. Prior to government maize provisioning, *T. esculentum* was a staple food for the Twe, but today, it has completely dropped out of the diet. We were initially puzzled by this observation because the Twe frequently encounter *T. esculentum* tubers, while encounters with *F. angustifolia* tubers and the two fruits listed in Table 3.5 are relatively infrequent. However, the low return rate coupled with the high energetic demands of procuring the tuber may well explain the Twe avoidance. If we assume that the Hadza expend similar amounts of energy while digging, the caloric acquisition rates for the some of the USOs reported in Vincent [36] would be much reduced, while others remain high (Table 3.6).

Our estimates of energy expenditure are not generalizable to all USOs. Seasonal variations in USO nutrient composition have implications for caloric content and return rates. Depending on the type of USO, weight and nutrient reserves will be highest at the beginning of the growing season, just before flowering or before the reproductive period. Return rates will be highest at these times. In addition, USO depth below the surface and soil type may affect energy expenditure during procurement. For example, consider *Lapeirousia sp.* grass corms. At Okau, these are available at the end of the rainy season. They are small and found only a few centimeters beneath the surface. At this time of year, the soil is relatively soft and children are able to procure the corms easily using small sticks. On average, it takes less than one minute to extract a corm, and we suspect that heart rate increases while digging are minimal. Both *T. esculentum* and *F. angustifolia* are much more difficult to obtain. They grow deeper below the surface in hard, rocky soils.

Soil qualities may vary geographically, affecting return rates for the same USO species within a region. Vincent [36] shows caloric returns for *Vigna fructescens* in two different habitats. In sand channels in *Grewia* bushland, it takes only 12 minutes to dig one kilo, while in brown loam soil of *Acacia* bushland, it takes 40. Soil qualities may also vary seasonally. In the regions surrounding Lake Eyasi, soils are very rich in clay [70], which may become cemented when wet, increasing digging costs during the rainy season. O'Connell

et al. [71] show that return rates of *Perideridia sp.* tubers respond to soil qualities as well. When the soil is very wet or very dry, the tubers are more difficult to acquire, and return rates are relatively low.

3.5 Conclusions

Our review of nutritional composition of African USOs suggests that nutritional quality is highly variable. However, USOs are typically low in fiber relative to other plant foods. Additionally, they are a good source of calories, carbohydrate, and sometimes protein. In this sense, many USOs are a high-quality food source. However, more work is needed to assess the nutritional composition of wild African USOs. The current data do not provide sufficient detail on insoluble fiber content to estimate the digestible energy content. Detailed information on carbohydrate composition would better inform estimates of the digestibility of raw USOs. Future work should also assess within-species seasonal and geographic variation in nutritional composition.

The energetic costs associated with digging USOs can be considerable. Most optimal foraging models make the simplifying assumption that procurement costs are the same for all resource types. However, if digging costs are factored into caloric acquisition rates, many USOs provide substantially fewer calories per hour than previously assumed. This reduction in profitability is further exacerbated when return rates are calculated using metabolizable energy rather than gross energy content. Our data suggest that despite high biomass and abundance in many environments, low-calorie USOs may not provide sufficient caloric returns to provision a forager and her dependents. However, many USOs have a calorie content comparable with other wild plant foods, and have very high return rates even considering energy expenditure. Those with a higher calorie content, or those beneath soft soils, can provide very high rates of caloric acquisition. We suggest that accounting for energy expenditure in calculated return rates will give a more accurate picture of the desirability of USOs relative to other food items. A larger sample size of heart rate data collected in different seasons is desirable.

Compared with meat, USOs are a reliable, low variance resource [25]. Other work suggests that protein was not limited in hominin diets [18, 21]. Neither meat from large ungulates nor USOs are an adequate source of fat, but both have relatively high caloric return rates. We find that energy expenditure is high during USO acquisition, but the costs of tracking, hunting, and transporting large game or competitive scavenging are potentially higher. Further, digging technology is simple, and early stone tools do not

suggest an advantage in capturing large game. Even with modern poisoned bow and arrow technology, hunting success is low among hunter gatherers [26]. The archaeological evidence for the occasional inclusion of animal foods in Plio-Pleistocene hominin diets is indisputable, but the lack of evidence for USO consumption is likely inconsequential. Plant foods do not preserve well in archaeological contexts, and several lines of evidence support the hypothesized importance of USOs [40].

Table 3.1: Nutrient content of selected African underground storage organs

taxon/type	Local	kcal/100g	ME[1]	water	protein[2]	fat[3]	starch	NSC[4]	carb[5]	fiber	NDF[6]	source
Vigna frutescens T[7]	1	271	-	69	10	2	-	87	-	-	31	10
Vatoraea pseudilablab T	1	225	-	89	5	3	-	91	-	-	35	10
Rhynchosia comosa T[8]	1	174	-	79	14	1	-	83	-	-	49	10
Coccinia surantiaca T	1	337	-	87	14	0	-	83	-	-	13	10
Vigna macrorhyncha T	1	292	-	86	14	2	-	81	-	-	22	10
Dioscorea semperflorens ‡‡	2	-	-	70	5.4	0.1	-	-	-	-	-	11
Dioscorea mangenotiana ‡‡	2	-	-	68	9	-	-	-	-	-	-	11
Dioscorea praehensis ‡‡	2	-	-	-	7	0.6	-	-	-	-	-	11
Dioscorea burkilliana ‡‡	2	-	-	61	6	0.2	-	-	-	-	-	11
Dioscorea minutiflora ‡‡	2	-	-	69	5	-	-	-	-	-	-	11
Dioscorea demetorum ‡‡	2	-	-	-	9	1	-	-	-	-	-	11
Dioscorea preussi ‡‡	2	-	-	82	9	0.2	-	-	-	-	-	11
Dioscorea Bifera	2	-	-	68	6	0.5	-	-	-	-	-	11
Brachystelema sp. T	3	323	-	93	1	6	-	-	107	-		12
Coleus esculentus R	3	352	-	70	3	26	-	-	84	-	-	12
Cyanastrum johnstonii B	3	330	-	65	6	5	-	-	103	-	-	12
Dioscorea Bifera B	3	313	-	88	14	3	-	-	100	-	-	12
Dioscorea dumetorum R	3	327	-	86	40	20	-	-	64	-	-	12
Dioscorea cf. prahensilis R	3	325	-	74	11	12	-	-	94	-	-	12
Dioscorea schimperena R	3	323	-	77	1	1	-	-	110	-	-	12
Disa welwitschii T	3	350	-	78	1	9	-	-	103			12
Eriosema verdickii R	3	298	-	74	52	32	-	-	42	-	-	12
Eriosema sp R	3	308	-	59	12	3	-	-	101	-	-	12
Monadenium discoideum T	3	356	-	86	8	17	-	-	80	-	-	12
Modadenium herbaceum T	3	353	-	82	8	32	-	-	79	-	-	12
Monadenium simplex T	3	330	-	79	5	14	-	-	96	-	-	12
Nymphaea caerulea R	3	332	-	89	6	6	-	-	104	-	-	12
Nymphaea maculata R	3	340	-	61	6	9	-	-	100	-	-	12
Nymphoides brevipidecellata R	3	325	-	89	10	12	-	-	94	-	-	12

Table 3.1: Continued

taxon/type	Local	kcal/100g	ME[1]	water	protein[2]	fat[3]	starch	NSC[4]	carb[5]	fiber	NDF[6]	source
Sphenostylis stenocarpa T	3	334	-	74	6	1	-	-	108	-	-	12
Albuca canadensis B‡‡*	4	-	-	77	0.6	0.1	-	-	-	-	-	13
Cyperus usitatis B‡‡*	4	-	-	76	2.5	0.6	-	-	-	-	-	13
Pelargorium sidoides B‡‡*	4	-	-	60	5	0.1	-	-	-	-	-	13
Talium caffrum T‡‡*	4	-	-	80	1	0.1	-	-	-	-	-	13
Nerine laticoma B	5	315	-	77	1	0.1	-	-	18	1	-	14
Annesorrhiza capensis T	5	340	-	77	0.3	0.2	-	-	19	2	-	14
Centella asiatica T	5	242	-	82	3	1	-	-	8	2	-	14
Chamarea capensis T	5	321	-	80	1	0.1	-	-	18	0.6	-	14
Bachysteima circinatum T	5	110	-	94	0.4	0.1	-	-	4	0.6	-	14
Ceropegia multiflora T	5	120	-	91	1	0.09	-	-	6	0.5	-	14
Ceropegia stemantha T	5	88	-	93	1	0.1	-	-	4	0.3	-	14
Fockea angustifolia T	5	103	-	91	0.5	0.2	-	-	5	1.4	-	14
Pentarrhinum insipidum T	5	192	-	85	4	0.5	-	-	7	2	-	14
Tenaris schutzel T	5	105	-	93	0.3	0.1	-	-	6	0.5	-	14
Commiphora angolensis T	5	-	-	76	-	-	-	-	-	-	-	14
Commiphora pyracanthoides T	5	187	-	81	-	-	-	-	-	-	-	14
Boscia albitrunca T	5	449	-	68	7	0.2	-	-	20	4	-	14
Ipomea welwitschii T	5	209	-	86	0.5	0.6	-	-	11	1	-	14
Coccinea adoensis T	5	289	-	82	1	0.1	-	-	16	0.6	-	14
Coccinea sessifolia T	5	205	-	84	2	0.1	-	-	10	2	-	14
Coccinea welwitschii T	5	305	-	78	3	0.2	-	-	15	3	-	14
Kedrostis africana T	5	226	-	84	3	0.1	-	-	10	3	-	14
Kedrostis hirtella T	5	321	-	78	3	0.1	-	-	16	1.4	-	14
Kedrostis nana T	5	65	-	94	0.2	0.1	-	-	4	1	-	14
Cyperus esculentus B	5	342	-	77	1	0.1	-	-	19	1	-	14
Cyperus fulgens B	5	569	-	65	1	0.2	-	-	32	1	-	14
Cyperus rotundus B	5	633	-	60	5	0.3	-	-	32	2	-	14
Mariscus indecorus B	5	-	-	62	-	-	-	-	-	-	-	14

Table 3.1: Continued

taxon/type	Local	kcal/100g	ME[1]	water	protein[2]	fat[3]	starch	NSC[4]	carb[5]	fiber	NDF[6]	source
Dioscorea dumetorum T	5	540	-	65	0.5	0.1	-	-	31	1	-	14
Dioscorea elephantipes T	5	98	-	93	0.3	0.1	-	-	5	1	-	14
Jatropa erythropoda T	5	183	-	87	1	0.2	-	-	9	2	-	14
Manihot utilissima T	5	101	-	93	0.3	0.1	-	-	6	1	-	14
Elephantorrhiza elephantina T	5	208	-	78	1	0.2	-	-	17	3	-	14
Tylosema esculentum T	5	125	-	91	0.6	0.1	-	-	7	2	-	14
Tylosema fassoglense T	5	237	-	80	2	0.5	-	-	11	4	-	14
Spiloxene azautica B	5	348	-	78	1	0.4	-	-	19	1	-	14
Babiana curviscapa B	5	36	-	57	5	0.3	-	-	36	0.6	-	14
Babiana dregei B	5	655	-	60	2	0.3	-	-	36	0.4	-	14
Babiana hypogea B	5	-	-	79	-	-	-	-	-	0.4	-	14
Babiana mucronata B	5	405	-	76	0.7	1.3	-	-	21	1	-	14
Babiana pubescens B	5	675	-	59	2	0.4	-	-	37	0.5	-	14
Lapeirousia sandersonii B	5	859	-	47	2	0.1	-	-	49	0.6	-	14
Monea fugax B	5	857	-	48	2	0.4	-	-	48	1	-	14
Monea serpentina B	5	955	-	42	7	0.5	-	-	48	1	-	14
Monea unguiculata B	5	799	-	51	1	0.3	-	-	28	0.6	-	14
Watsonia pyrimidata B	5	500	-	69	1	0.3	-	-	28	0.6	-	14
Allium dregeanum B	5	553	-	65	3	0.1	-	-	30	1	-	14
Dipendi platyphyllum B	5	281	-	82	1	0.1	-	-	16	0.4	-	14
Dipendi rigidifolium B	5	199	-	87	1.3	0.02	-	-	11	0.3	-	14
Dipendi viride B	5	215	-	86	1	0.1	-	-	12	0.3	-	14
Dracaena hookerana B	5	266	-	82	1.4	0.5	-	-	13	2	-	14
Eriospermum parvifolium B	5	153	-	88	0.5	0.1	-	-	8	2	-	14
Ledebouria revoluta B	5	333	-	77	2	0.2	-	-	2	0.4	-	14
Ledebouria luteola B	5	519	-	66	4	0.4	-	-	26	3S	-	14
Sansevieria pearsonii T	5	105	-	89	1	0.1	-	-	5	4	-	14
Conicosea pugioniformis T	5	397	-	73	0.6	0.2	-	-	23	1	-	14
Rushia rigens T	5	269	-	75	3	0.1	-	-	13	8	-	14

Table 3.1: Continued

taxon/type	Local	kcal/100g	ME[1]	water	protein[2]	fat[3]	starch	NSC[4]	carb[5]	fiber	NDF[6]	source
Nymphaea caerulia R	5	174	-	86	1	0.2	-	-	9	2	-	14
Eulophia hereroensis B	5	105	-	93	0.6	0.1	-	-	5	0.5	-	14
Oxalis annae B	5	851	-	48	6	0.2	-	-	45	0.5	-	14
Oxalis pes-caprae B	5	478	-	69	0.2	0.04	-	-	26	2	-	14
Cyanella hyacinthoides B	5	736	-	56	4	0.6	-	-	39	1	-	14
Campthorrhiza sp. C	6	429	390	40	7	2	73‡	80	-	1.5	3	15
Fockea angustifolia T	6	385	302	90	7	7	3‡	57	-	8	22	15
Lapeirousia sp. C	6	411	333	40	5	7	0.3‡	65	-	6	17	15
Tylosema esculentum T	6	438	361	75	11	4	11‡	34	-	17	42	15

[1] T=tuber, B=bulb, R=rhizome, C=corm
1=Lake Eyasi, Tanzania, Hadza; 2=Gabon, Central African Republic, Cameroon, rainforest, Aka, Baka, Bakola;
3=Zambezian Woodland, Southern Africa; 4=Upper Karoo, South Africa; 5=Southern Africa; 6=Kunene, Namibia, Twe
10=Crittenden, 2009 [72]; 11=Hladick and Dounias, 1993 [**?**]; 12=Malaisse and Parent, 1985 [73]; 13=Youngblood [74], 2004;
14=Wehmeyer, 1986 [75]; 15=Chapter 2
[1] Metabolizable Energy (g/100g). This is an estimate of the digestible energy in the raw USO, based on the fiber content

[2]calculated using 4kcal/gram, unless otherwise noted

[3]calculated using 9kcal/gram, unless otherwise noted

[4]nonstructural carbohydrate

[5]calculated using 4kcal/gram, unless otherwise noted

[6]Neutral Detergent Fiber (NDF) is nongistible fiber. NDF inhibits bioavailability of total energy, fat, and protein.
Values are reported as a percent of total fiber.

[7] average of four specimens

Table 3.1: Continued

[8] average of two specimens

* these are raw, fresh values, not dry weight

‡‡Protein, fat, carbohydrate, and fiber values are reported as percent

‡These data were generated for the current study, and do not appear in Leonard et al., 2015.
See methods section for a description of the analysis.

Table 3.2: Nutrient content of fruits targeted by Hadza and Twe

species	location	moisture (%)	crude fiber (%)*	NDF(%)[1]	protein (g/100g)	fat (g/100g)	NSC[2](g/100g)	Gross kcal[3]	Metabolizable kcal[4]	Source
Acacia nilota	Lake Eyasi, Tanzania	68		30	27	0.2	37	267		Crittenden, 2009
Adansonia digitata seed	Lake Eyasi, Tanzania	5	14*		36	29		454		Murray et al., 2001
Adansonia digitata pulp	Lake Eyasi, Tanzania	5	45*		3	1		203		Murray et al., 2002
Adansonia digitata pulp†	Lake Eyasi, Tanzania	57		16	3.5	1	78	337		Crittenden, 2009
Berchemia discolor	Kunene, Namibia	40	7	14	26	13	311	432	350	Leonard et al., 2015
Cordia cf sinensis	Lake Eyasi, Tanzania	73	12*		13	2		342		Murray et al., 2015
Cordia cf sinensis	Lake Eyasi, Tanzania	60		39	12	3	37	232		Crittenden, 2009
Diospyros mespiliformis	Kunene, Namibia	68	13	35	7	10	195	440	274	Leonard et al., 2015
Ficus sycomorus	Kunene, Namibia	-	19	37	29	9	196	447	298	Leonard et al., 2015
Ficus sycomorus	Lake Eyasi, Tanzania	24	-	29	3	10	54	329		Crittenden, 2009
Grewia bicolor	Lake Eyasi, Tanzania	26	13		12	2		330		Murray et al., 2015
Grewia villosa	Lake Eyasi, Tanzania	24	13*		7			337		Murray et al., 2016
Hyphaene petersiana	Kunene, Namibia	-	33	42	18	8	106	444	210	Leonard et al., 2015
Salvadora persica	Lake Eyasi, Tanzania	55		13	11	18	48	447		Crittenden, 2009
Sclerocarya birrea	Lake Eyasi, Tanzania	83	38*		4			232		Murray et al., 2018

[1]neutral detergent fiber (insoluble fiber)
[2]nonstructural carbohydrate
[3]neutral detergent fiber (insoluble fiber)
[4]total energy
[5]digestible energy portion

Table 3.3: Average return rates of Twe underground storage organs and fruits

plant	kcal/hour	number of observations
B. discolor (fruit)	2515	3
D. mespiliformis (fruit)	5297	3
F. angustifolia (tuber)	4555	2
Lapeirousia sp. (corm)	167	5
T. esculentum (tuber)	933	2

Return rates are an average of the total number of observations listed in the final column.

Table 3.4: Twe heart rate data for digging underground storage organs and picking fruits

participant age/sex	taxon	collection type	heart rate during search	kcal/min during search	heart rate during collection	kcal/min during collection
18/male	*T. esculentum* (tuber)	digging	70	4	118	11
18/male	*F. angustifolia* (tuber)	digging	85	6	126	12
30/female	*D. mespiliformis* (fruit)	picking	86	0.9	99	2

The Twe dig *T. esculentum* tubers using a metal rod or hoe if possible, but use a simple wooden digging stick for *F. angustifolia* tubers. Ripe *D. mespiliformis* fruits are collected directly from the tree or from the ground beneath it.

Table 3.5: Return rates taking into account estimated energy expenditure and metabolizable energy content

taxon	gross kcal/hour[a]	gross kcal/hour − energy expenditure[b]	metabolizable kcal/hour [c]	metabolizable energy/hour − energy expenditure
B. discolor (fruit)	2515	2449	2287	2221
D. mespiliformis (fruit)	5297	5231	3848	3782
F. angustifolia (tuber)	4555	4195	3700	3310
T. esculentum (tuber)	933	513	652	232

[a]This is the total number of calories acquired per hour (Table 4.8)
[b]This value is the total number of calories minus the extra energy expended during collection.We subtracted energy expenditure during an hour of search from energy expenditure during an hour of collection to calculate the net increase in energy expenditure during an hour of collection for each plant. For *B. discolor* and *D. mespiliformis* 66kcal/hour, *F. angustifolia* 390kcal/hour,*T. esculentum* 420kcal/hour
[c]This takes into account limited digestibility of nutrients based on the indigestible fiber content. See Chapter 2 for a detailed explanantion

Return rates are an average of the total number of observations listed in the final column.

Table 3.6: Net return rates of Hadza underground storage organs, based on published data[1]

taxon	kcal/hour	net kcal/hour[‡]
Vatovaea pseudolablab	1816	1426
Vigna fructescens, sand channels	3240	2850
Vigna fructescens, acacia woodlands	1077	687
Vigna macrorhyncha	1967	1577
Vigna sp.	884	494

[1]from Vincent, 1985
[‡]calculated using the average energy expenditure for Twe tubers, 390kcal/hour

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CHAPTER 4

PLANT MICROREMAINS IN DENTAL CALCULUS AS A RECORD OF PLANT CONSUMPTION: A TEST WITH TWE FORAGER-HORTICULTURALISTS

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Plant microremains in dental calculus as a record of plant consumption: A test with Twe forager-horticulturalists

Chelsea Leonard [a,b,*], Layne Vashro [b], James F. O'Connell [b], Amanda G. Henry [a]

[a] Plant Foods in Hominin Dietary Ecology Research Group, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
[b] Department of Anthropology, University of Utah, Salt Lake City, UT, United States

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ABSTRACT

Starch granules and phytoliths trapped in dental calculus preserve a record of plant consumption. Analysis of these microscopic plant remains has increased in popularity in recent years, providing information on diet that complements dental microwear and stable isotope studies. However, it is unclear how accurately these microremains reflect plant consumption. This study examines how well starch granules and phytoliths in dental calculus from a living population (the Twe) with a well-documented diet capture the range and intensity of plant consumption. We find that plant microremains are a poor predictor of plant consumption on an individual level, but may provide a good signal of plant consumption across a population, as well as evidence for plant processing in the mouth. This is the first study to test how well plant microremains in dental calculus reflect plant consumption in a population with a known diet. Results from this project have implications for interpreting plant microremain data from archaeological dental calculus samples.

1. Introduction

Starch granules and phytoliths in dental calculus are increasingly used as dietary markers in archaeological investigations. Direct signatures of ancient plant consumption are rare in archaeological contexts, but plant microremains in dental calculus have helped to elucidate diets in many contexts, ranging from the early consumption of domesticates in the Holocene (cf. Henry and Piperno, 2008; Mickleburgh and Pagan-Jimenez, 2012; Li et al., 2010; Piperno and Dillehay, 2008) to early hominin plant consumption (Henry et al., 2011, 2012; Henry et al., 2014; Salazar-García et al., 2013). Other direct measures of plant consumption such as carbon stable isotopes or tooth microwear analysis provide only general information on categories of plants consumed or the physical properties of those plants. Plant microremains like starches and phytoliths can be taxonomically distinct, and their presence in dental calculus sometimes reveals the consumption of specific plant families or genera.

Despite promising results in many time periods and geographic regions, we have yet to determine exactly what type of dietary signal plant microremains in dental calculus record. For example, some authors suggest that a high incidence of starches and phytoliths from certain plants indicates that those plants were consumed at high frequency (Henry and Piperno, 2008; Middleton and Rovner, 1994; Piperno and Dillehay, 2008). Others draw comparisons between individuals or groups based on the numbers of plants represented by microfossils (Dudgeon and Tromp, 2012; Henry et al., 2014; Mickleburgh and Pagan-Jimenez, 2012). While such comparisons are logically appealing, we do not yet understand the mechanism for preservation of microremains in dental calculus. It is recognized that calculus formation rates vary among individuals (Jin and Yip, 2002; White, 1997), and some researchers have acknowledged this as a potential source of variation in the preservation of microremains (Henry et al., 2014). However, the extent to which individual differences in calculus formation might create individual variation in the microremain record is unclear. Starches appear to be more plentiful than phytoliths in modern human dental calculus (Boyadjian et al., 2007; Fox et al., 1994, 1996; Henry and Piperno, 2008; Juan-Tresserras et al., 1997; Scott Cummings and Magennis, 1997), possibly because humans preferentially eat starchy foods, but we do not know what other biases may exist in the dental calculus record.

Here we present the first comparison of diet and plant microremains in dental calculus from a living population with a well-documented diet. We report on the relationship between plant consumption and plant microremains in dental calculus from Twe forager-horticulturalists in order to characterize the preservation of plant microremains in human dental calculus. Our analyses address the following questions: 1. Is diet consistently recorded across all individuals in the same population, given their similar diet? 2. Do plant microremains in Twe dental calculus reflect the range of plants consumed? 3. Is starch quantity in dental calculus proportional to dietary concentration? Initial results suggest that starches and phytoliths do record diet, but that the relationship between diet and microremains preserved in calculus is not as straightforward as previously assumed.

* Corresponding author at: Department of Anthropology, University of Utah, 270 S 1400 E, Salt Lake City, UT 84112. Tel.: (801) 581 6251.
E-mail address: chelsea.leonard@anthro.utah.edu (C. Leonard).

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1.1. Background: plant microremains and dental calculus

Plant microremains are microscopic plant residues with taxonomically specific diagnostic features. Microremains include but are not limited to starch granules, phytoliths, diatoms, spores, and pollen granules. This paper discusses only starch granules and phytoliths. Starch granules are comprised of complex carbohydrates and are formed in plant tissues for energy storage. Starches are formed in specialized plant organelles called amyloplasts. Starch granule formation begins at a central point called the hilum, and continues with alternating layers of amylose and amylopectin. The alternation of amylose and amylopectin results in a semi-crystalline structure which gives starch some unique properties, such as a polarization cross under cross-polarized light (Barton and Fullagar, 2006; Field, 2006; Gott et al., 2006). Plants produce two types of starch, transient and reserve starches. Transient starch is formed for short-term energy storage in photosynthetic tissues like leaves, while reserve starch is formed for long-term energy storage in plant storage organs, fruits, and seeds (Gott et al., 2006; Henry, 2012; Sivak and Preiss, 1998). Transient starch morphologies are simple and of limited use in dental calculus studies (Shannon et al., 2009). Reserve starch morphologies may vary along taxonomic lines (Reichert, 1913; Torrence, 2006), but also within species and within individual plants. Surface features like the presence and placement of the hilum, striations called lamellae, cracks, and fissures, as well as the shape and symmetry of the polarization cross are used to distinguish among starches from different taxa (Torrence et al., 2004; Torrence and Barton, 2006).While starch can survive for thousands of years in certain conditions, heat and moisture cause starches to gelatinize, and acidic conditions and enzymatic activity also damage starches. Dental calculus provides a protective environment that facilitates starch survival (Henry, 2012).

Phytoliths are microscopic noncrystalline silica bodies that are formed in and between plant cells when soluble silica from the ground water precipitates into plant tissues (Henry, 2012; Pearsall, 2000; Piperno, 2006). Phytoliths provide structural support and defense against herbivory (Weiner, 2010). Many plants produce phytoliths, and phytolith production is largely under genetic control, such that phytolith-producing plants tend to occur in the same families, genera, and species, regardless of region of origin (Bamford et al., 2006). Environmental conditions including the soil temperature and water content, concentration of monosilicic acid in the soil, soil pH, and climate can also affect phytolith production (Madella et al., 2002; Piperno, 1988). Phytolith concentration is highest in leaves, husks, rinds, bark, and fruits (Piperno, 2006; Rovner, 1983). Phytolith morphologies are often taxonomically distinct, and may also reflect the specific plant tissue in which they form (Tsartsidou et al., 2007). Diagnostic features include size, shape, texture, and ornamentation (Madella et al., 2005; Piperno, 2006). Phytoliths are soluble in basic conditions (Rovner, 1983), but may persist for millions of years (see Prasad et al., 2005). The oldest phytoliths recovered from dental calculus date to at least 2 Ma (Henry et al., 2012).

Dental calculus provides a protective environment where starches and phytoliths can survive for thousands of years (Henry et al., 2011). Dental calculus is mineralized plaque which forms both above and below the gingival margin. In this project we consider only supragingival calculus deposits, as calculus was recovered from living people. Supragingival calculus deposits form preferentially near the salivary glands in the mouth, on the lingual sides of mandibular incisors and the buccal sides of maxillary molars and premolars (Jin and Yip, 2002; Bergström, 1999). Supragingival calculus forms when plaque on tooth surfaces is bathed in calcium and phosphate rich saliva (Jin and Yip, 2002; Lieverse, 1999). The rate of mineralization varies among individuals according to age, oral hygiene, and possibly diet (Bergström, 1999; Lieverse, 1999). Smoking increases the rate of calculus formation (Bergström, 1999). Dental calculus is 80% inorganic, comprised of calcium phosphate in various phases, including hydroxyapatite, brushite, whitlockite, and octacalcium phosphate (Abraham et al., 2005; Lieverse, 1999). Older deposits tend to be richer in hydroxyapatite, while both young deposits and supragingival calculus are richer in brushite (Schroeder and Bambaur, 1966). The organic portion of calculus includes bacteria, DNA, lipids, proteins, pollen, phytoliths, and starch granules (Hillison, 1996; Lieverse, 1999; Warinner et al., 2014).

Due to individual variability in the amount and rate of calculus formation, we cannot assume a simple relationship between plant consumption and plant representation in dental calculus deposits. We know that starch granules are more common than phytoliths in human dental calculus (Boyadjian et al., 2007; Fox et al., 1994, 1996; Henry and Piperno, 2008; Juan-Tresserras et al., 1997; Scott Cummings and Magennis, 1997), but we do not know what factors bias starch preservation in dental calculus. For example, population-level or individual differences in salivary amylase copy number may correspond to differences in amylase activity (Perry et al., 2007). This in turn could affect the rate of starch digestion in the mouth, which combined with variable calculus formation rates complicates our understanding of starch incorporation. Below we assess how reliably Twe plant consumption is recorded in Twe dental calculus.

1.2. Background: the Twe

The Twe are a group of forager-horticulturalists who live in Northwestern Namibia and Southwestern Angola. Culturally they resemble the well-studied Himba pastoralists, but most Twe do not own animals and make a living by foraging and gardening (Vashro, 2014). The Twe live in an arid, mountainous environment with marked seasonality. During the rainy season, many Twe grow maize (*Zea mays* L.) which they dry and grind into a course meal, as well as pearl millet (*Pennisetum glaucum*(L.)), squash (*Cucurbita* sp.), melons (Cucurbitaceae, several species), and sugarcane (*Saccharum* sp.). The Twe also collect several wild foods during the rainy season, most notably *Berchemia discolor* (Klotzsch) Hemst. (bird plum) berries which are eaten in large quantities. Gardening is not possible during the dry season, and the Twe rely on dried maize meal and foraged foods including fruits from *Hyphaene petersiana* Klotzsch ex Mart. (makalani palm) and *Diospyros mespiliformis* L. (jackalberry), as well as various underground storage organs. Since the end of 2007 the Twe have received subsidies of maize meal from the Namibian government, as well as small herds of goats, which produce a limited amount of sour milk. Today the Twe are heavily reliant on the government maize meal subsidies, but still garden and regularly collect a wide range of wild plant foods.

The Twe are semi-mobile. Most people have a 'home' where they spend much of their time, but they also move to different compounds around the region and occasionally visit friends and families in distant locations where different foods may be available. This work focuses on Twe living at a government camp called Otjomoru in the Zebra Mountains and the nearby traditional settlement Okau, and Twe living at a government camp near Epupa Falls called Ohayuua. These camps are considered 'home' locations by many people due to the availability of government maize subsidies. The Twe do not have any access to dental care. Many people occasionally chew on a specific type of stick (called 'omundumise' in Herero), but no one uses toothbrushes, toothpaste, or dental floss, and there is no access to dental care and very limited access to medical care.

2. Methods

One of us (CL) stayed with the Twe in July–October 2012 (dry season) and April–May 2013 (late rainy season) in order to collect dental calculus samples, dietary information, and samples of plant foods. This project was approved by the University of Utah Institutional Review Board, the Namibian Ministry of Health and Social Services, and the Namibian Ministry of Environment and Tourism, and was conducted with a Namibian Research Visa. All work was conducted using an interpreter who is a native Otjiherero speaker. Participants

Table 1
Most commonly consumed Twe plant foods.

Plant name	Plant part consumed	Preparation
Gardened		
Zea mays L. [maize]	Kernels	Dried, ground, boiled with water to create porridge
Pennisetum glaucum L. [pearl millet]	Grains	Dried, ground, boiled with water to create porridge
Arachis hypogaea L. [peanut]	Nut	Unknown
Citrullus lanatus (Thunb.) Mastumura & Nakai [watermelon]	Fruit	Raw
Cucurbita sp. [pumpkin, several species]	Fruit, leaves	Boiled
Cucurbitaceae, other species [melon]	Fruit	Raw
Daucus sp. [carrot]	Taproot	Unknown
Fabaceae sp. [beans, species unknown]	Bean	Boiled
Ipomoea sp. [sweet potato]	Tuber	Boiled
Opuntia sp. [prickly pear cactus]	Fruit	Raw
Saccharum sp. [sugarcane]	Stalk	Raw
Solanum sp. [tomato]	Fruit	Raw
Foraged		
Berchemia discolor (Klotzsch) Hemst.	Berries	Raw, often dried
Diospyros mespiliformis L.	Fruit	Raw, often dried
Ficus sycomorus L. [fig]	Fruits	Raw or dried and ground, cooked as porridge
Fockea angustifolia K. Schum	Tuber	Raw
Grewia sp. [ozohamati]	Berries	Raw
Grewia sp. [ozombapu]	Berries	Raw
Grewia tenax (Forssk) Fiori [ozonjenjere]	Berries	Raw
Hyphaene petersiana Klotzsch ex Mart. [Makalani palm]	Nut	Raw or ground and cooked as porridge
Lapeirousia sp. [ozondungua]	Corms	Roasted for approximately 5 minutes to remove tunic
Unknown species [ombowa/ garden weed]	Leaves	Boiled
Unknown species [omundumise/toothbrush stick]	Stick	Chewed raw to clean teeth
Unknown species [ozonduvi]	Corms	Roasted for approximately 5 minutes
Unknown species [otjihakariwa]	Tuber	Raw
Tylosema esculentum A. Schreib	Bean and rhizome	Bean preparation unknown, rhizome roasted for an hour or more
Ximenia americana L.	Fruit	Raw

Plant species are listed alphabetically, with the exception of *Z. mays* L. and *P. glaucum* L. These are highlighted to indicate that they are the most important contributors to the plant portion of the Twe diet.

were selected based on presence of calculus deposits, targeting equal numbers of men and women in age groups of 20–29, 30–39, 40–49, 50–59, 60–69, and 70 + years. Participants were informed about the nature of the study and were compensated for their time.

2.1. Diet

Information on diet was collected using a variety of methods. 1) We interviewed each participant at the time of dental calculus sampling. They were asked about the range of gardened foods in the diet, the range of foraged foods in the diet, the range of commercial foods in the diet, seasonal changes in diet, the frequency of meat consumption, how diet responds to drought, differences in wild plant availability in different locations, the frequency and duration of trips away from the 'home' location, and whether or not the participant smokes (see Supplement 1 for a list of questions). 2) We conducted 24-hour diet recalls opportunistically with each participant. The participant listed the number of meals and foods consumed during the preceding 24-hour period. 3) We conducted camp scans where we walked past each compound at meal times and noted what people were eating and the approximate quantities.

Table 2
Starch content of starch containing foods in the Twe diet.

Plant	Starches/mg
Adansonia digitata (baobab)	5116.77
Cucurbita sp.	8433.5
Fockea angustifolia K. Schum	294.45
Lapeirousia sp.	4988.99
Otjihakariwa	616.68
Ozonduvi	Negligible
Pennisetum glaucum L. (pearl millet)	922.13
Tylosema esculentum A. Schreib	300.01
Ximenia americana L.	27.78
Zea mays L. (maize)	4366.23

Starch contents listed are per milligram dry plant matter.

Table 3
Phytolith content of the phytolith producing foods in the Twe diet.

Plant	Phytoliths/% dry weight
Cucurbitaceae, unidentified melon fruit	8.19
Ficus sycomorus L.	4.02
Grewia tenax (Forssk.) Fiori	Negligible
Hyphaene petersiana Klotzsch ex Mart. fruit	5.14
H. petersiana leaf	9.41
Omundumise	0.109
Ozombapu	Negligible
Ozonjenjere	Negligible

Phytolith content is listed as the percent of total plant dry weight.

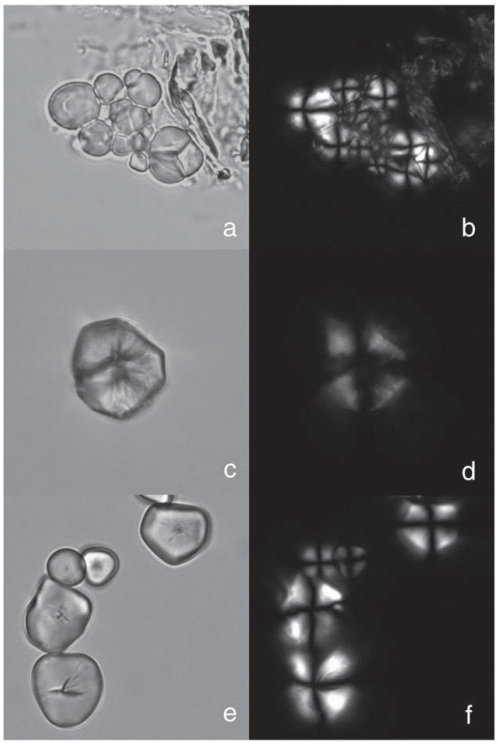

Fig. 1. Most common starch types in starch producing domesticates in the Twe diet. All images are shown at the same scale. Each box is 50 μm on a side. Images are paired; the first of each pair is shown under bright-field light and the second under cross-polarized light. a–b, *Cucurbita* sp. flesh, small granules, both simple and compound, tend to form in aggregates (a) or occasional compounds, with distinct crosses with thin, curved arms (b); c–d, *Pennisetum glaucum* L. seeds, polygonal granule with a deep stellate fissure, textured surface with radial cracks (c), and characteristic cross with wide, straight arms; and e–f, *Zea mays* L. kernels, characteristic polygonal granules with marked hilum, single or three-armed fissures and visible radial cracks (e) and distinct crosses with bending arms (f).

The following analyses rely on information collected during interviews. 24-hour diet recalls and camp scans were used to gain a general idea of the frequency and relative proportions of common foods in the diet.

2.2. Dental calculus collection

We collected dental calculus from the bucco-mesial surface of the bottom left canine (the Twe remove their mandibular incisors, and often have thick calculus deposits on their canines that extend toward the midline) of 74 participants between the age of 25 and 83, using a new autoclaved dental scaler for each person. The sample consists of roughly equal numbers of men and women of each age (see Supplement 2 for data spreadsheet, which includes participant information). Participants were asked to clean their teeth with a sterile single-use toothbrush, and we cleaned the teeth further by removing plaque before sampling calculus. We removed as much calculus as possible, but in some cases were not able to remove an entire deposit. Samples were stored in aluminum foil inside of plastic micro-centrifuge tubes. At the time of sampling, small bowls of water were placed in the workspace and saved in order to control for airborne contaminants. Calculus samples were weighed to the nearest tenth milligram using a Denver Instruments APX-260 scale.

2.3. Plant reference collection

When possible, specimens of each of the plant foods mentioned in interviews were collected with the help of Twe guides. Samples of all but three of the foraged foods were collected, but many gardened foods were not available because we worked with the Twe during a poor rainy season when gardening was not feasible for most people. However, we did collect the 'staple' gardened foods, which are maize (*Z. mays*), pearl millet (*P. glaucum*), pumpkin (*Cucurbita* sp.), and sugarcane (*Saccharum* sp.). All plants were cleaned with water and dried with silica gel beads in plastic sample bags. Voucher specimens were dried in a plant press and identified at the Namibian Botanical Research Institute (NBRI). Not all specimens were identifiable at NBRI. We refer to unidentified plant specimens by the Herero names used by the Twe.

Slides for starch description were prepared by directly mounting ground dry plant material on slides with 15 μl of distilled water and 15 μl of 25% glycerol. Starches were observed at 400× magnification using a Zeiss Axioscope microscope. At least fifty starches were photographed and measured, and a written description was generated for each plant following the terminology of the ICSN (2011). Most plants were collected and observed for starches in both 2012 and 2013 from different locations within the Twe range. We calculated the number of starch granules per milligram dry weight for each plant by mounting 1 mg dried, gently ground plant material suspended in water, and then counting the number of starches in 9 randomly chosen fields of view on each slide, using an in-house random number generator designed to identify one random field of view within each of nine concentric squares on a 22 × 22 mm microscope cover glass slip. This provided a random sample of all of the starches present on 68% of the slide, from which we could then calculate the number of starches in the total sample.

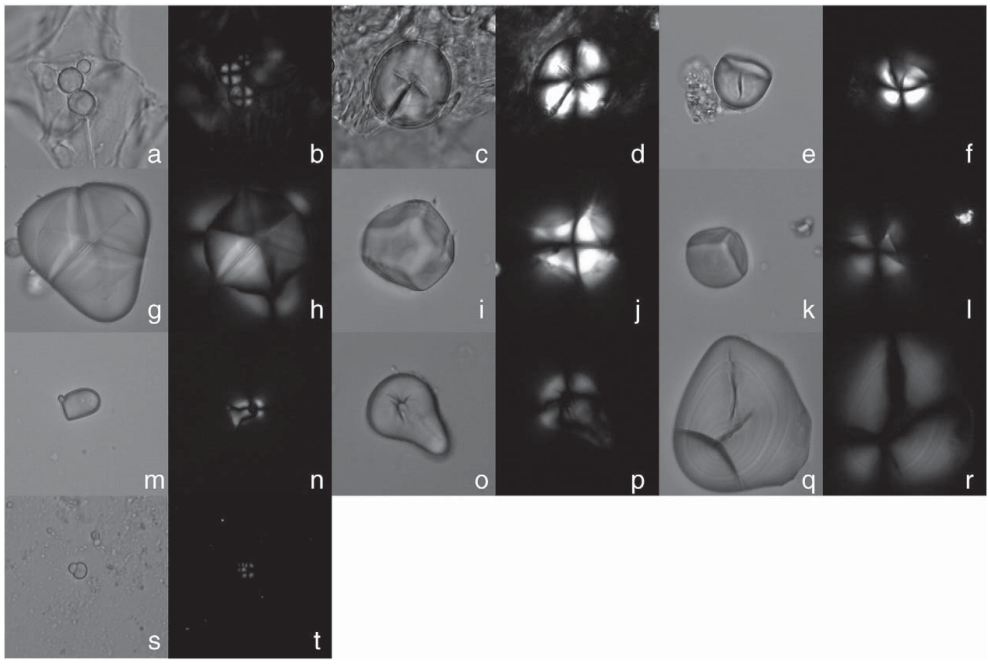

Fig. 2. Most common starch types in starch producing wild plants in the Twe diet. All images are shown at the same scale. Each box is 50 μm on a side. Images are paired; the first of each pair is shown under bright-field light and the second under cross-polarized light. a–b, *Adansonia digitata* L. fruit, small spherical granules (a) with centric, straight-armed crosses (b); c–f, *Fockea angustifolia* K. Schum tuber, spherical granules with deep radial fissures and lamellae (c) and interrupted straight-armed cross (d) and plano-convex granules (e) with radial fissure and interrupted cross with cured arms (f); g–h, *Lapeirousia* sp. corms, tripartite compound granules with eccentric long hilum and radial cracks (g) with indistinct, asymmetric crosses (h); i–l, otjihakariwa tuber, large polygonal granule (i) with thin-armed, curved cross (j) and rounded polygonal granule (k) with wide armed cross (l); m–n, ozonduvi corms, elongate hemispherical granule with refractive hilum (m) with wavy-armed cross (n); o–r, *Tylosema esculentum* A. Schreib tuber, pyriform granule (o) with interrupted cross (p), and large ovoid polygonal granule with distinct lamellae and radial cracks (q) with curved-armed cross (r); and s–t, *Ximenia Americana* L. fruit, small unequal compound granules (s) with symmetric straight-armed crosses (t).

Phytoliths were extracted by digesting between 100 and 500 mg dried plant material in nitric acid. The remaining material was weighed to calculate phytolith content as a percent of dry weight. A heating block was used to speed the reaction, and potassium chlorate was added to further accelerate the process. Phytoliths were weighed and mounted on slides using Permount. Phytoliths were photographed, measured, and described following the ICPN (Madella et al., 2005).

2.4. Dental calculus analysis

Each dental calculus sample was dissolved in 10% HCl for up to 2.5 h. We first tested this concentration of HCl on raw maize and millet starch for up to 24 h to ensure that starches would not be damaged. Each sample was centrifuged in a Thermo Scientific Heraeus Megafuge 16 centrifuge at 3500 rpm for 7 min, and rinsed twice with distilled water. Supernatant was removed until only 30 μm remained. This sample was then mounted on slides with 25% glycerol and observed at 400× magnification. Each microremain was photographed, measured, and compared with the plant reference collection. Starch identifications were made based on size, 2D and 3D shape, the placement of the hilum, presence or absence of lamellae, cracks, and fissures, and the placement and shape of the polarization cross. Many starches were not identified because they were nondiagnostic or because they were damaged to an extent that no diagnostic features remained. Phytoliths were identified based on size, shape, and surface features.

2.5. Statistical analyses

All analyses were conducted using R 3.1.0 (R Core Team, 2014) with the MASS package (Venables and Ripley, 2002). Figures were also generated using R 3.1.0. All models are described below. Missing data were excluded from all analyses (see Supplement 2 for data spreadsheet).

3. Results

3.1. Twe plant foods

The Twe regularly eat 31 cultivated and foraged plant foods (Table 1). This includes the foods that were mentioned in the majority of interviews. Other plants are included in the diet, but are less common. Maize porridge is the food most frequently consumed, followed by various foraged foods depending on the season and location. In the Zebra Mountains, *B. discolor* fruits are eaten in large quantities in the rainy season and *D. mespiliformis* fruits in the dry season. At Ohayuua, *D. mespiliformis* fruits are not available, but palm nuts (*H. petersiana*) are eaten frequently during the dry season. Palm nuts are not consumed in the Zebra Mountains, because participants say that they are not sweet enough. Gardened foods like melons and squash are frequently consumed during the rainy season in years with sufficient rainfall. Of the 31 commonly consumed plant foods, 11 contain starch and 8 contain phytoliths. Table 2 shows the number of starch granules per milligram dry weight. Table 3 shows the phytolith content as a percentage of total dry weight. Fig. 1 shows the most common starch types in starch producing domesticates, Fig. 2 shows the most common starch types in starch producing wild plant foods, and Fig. 3 shows the most common phytolith types.

3.2. Is diet consistently recorded across all individuals?

In general, older people and men had larger calculus deposits than young people (under 30/35) and women. This meant that it was not possible to collect samples from several young people and women, leading to a bias in our sample collection. It is possible that the factors controlling calculus formation in people with larger deposits also affect preservation of microremains within those deposits.

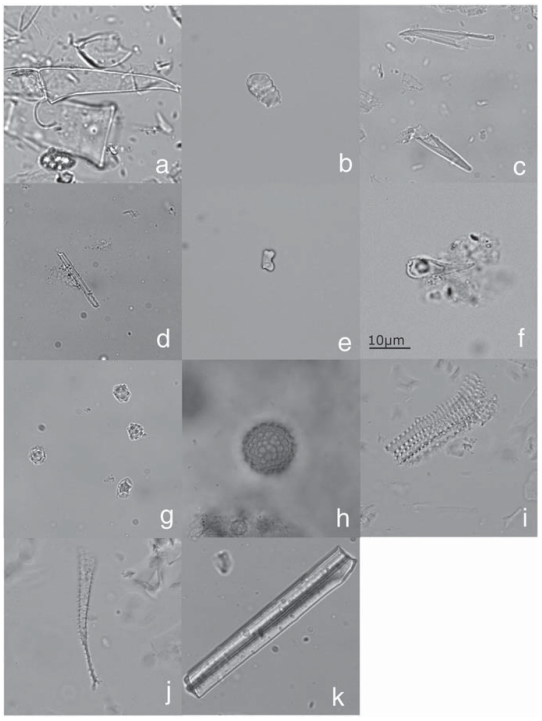

Fig. 3. Most common phytolith types in phytolith producing Twe foods. All images are shown under bright-field light at the same scale, and boxes are 100 μm on the side unless otherwise noted. a, *Cucurbita* sp. leaf, elongate lanceolate phytolith (possible hair cell); b, *Citrullus lanatus* (Thunb.) Matsumura & Nakai fruit, oblong laminate phytolith; c, *Ficus sycomorus* L. fruit, lanceolate hair cell; d, *Grewia* sp. (ozombapu) fruit, cylindrical elongate phytolith; e–f,*Grewia tenax* (Forssk.) Fiori fruit, (e) bilobate short cell, (f) acicular hair cell (box is 50 μm); g, *Hyphaene petersiana* Klotzsch ex Mart fruit, globular echinate phytolith; h,*H. petersiana* leaf, spherical granulate phytoliths; i–j, omundumise/toothbrush stick, twig (i) elongate dendritic phytolith, (j) lanceolate dendritic phytolith; and k, *Saccharum* sp. pith, elongate phytolith with three equally-spaced longitudinal ridges, forming a pointy triangle in cross-section.

Calculus samples vary in both the number of starches and the number of plants represented by starches. This variation is summarized in Tables 4 and 5. Many of the following analyses use the 'number of plants represented' as a variable. This variable includes only starch granules and phytoliths that were assigned to a taxon ("identified" starches and phytoliths). Unidentified starches and phytoliths were excluded from this analysis because we were interested in knowing how well the microfossils could record known diet. Most samples with identified starches or phytoliths contain only one identified plant (40%). In samples with only one plant represented, maize is the most common (42%). Fig. 4 shows examples of identifiable starches recovered from dental calculus.

Table 4
Summary of plant microremains recovered from Twe dental calculus.

	Mean	Standard deviation	Range	% samples present
Starch	8.27	8.50	0 to 48	98.64
Phytoliths	0.24	0.57	0 to 3	18.92
Number of plants represented by starch	0.77	0.97	0 to 4	n/a
Total number of plants represented	1.01	1.16	0 to 6	n/a

Table 5
Summary of starch granules recovered from Twe dental calculus.

	% samples	% total starch
Damaged starch	81.08	38.24
Unidentified (includes damaged and nondiagnostic starch)	93.24	71.24
Maize starch	31.10	9.64
Contain at least one plant	63.51	n/a
Contain more than one plant	21.62	n/a
Contain more than two plants	8.11	n/a
Contain more than three plants	4.10	n/a

Many starches (71%) were not assigned to a taxon, either because they were nondiagnostic or because they were unidentifiable due to damage. In our sample, damage to starches was not consistent with cooking (e.g. gelatinization of boiled maize starches), nor did it resemble damage from processing calculus samples in HCl. We suspect that some granules were damaged by salivary amylase in the mouth, but have not assessed this experimentally. All recovered phytoliths were identified to a taxon.

3.2.1. Individual differences based on age and sex

We estimated the effects of age and sex on the total number of starches per calculus sample. These analyses use negative binomial regression because the dependent variable is overdispersed count-data. Calculus collected from both men and older individuals contained more starches, but when we controlled for sample weight, the effects of age and sex disappeared (see Table 6). Because we are ultimately interested in the number of plants represented in each sample, we repeated this analysis with the total number of plants per sample and the total number of starchy plants as variables. In each case, neither age nor sex had strong or significant affects, but sample weight is a strong predictor of the total number of starches (see Table 6).

We also expected a sex difference in the preservation of palm leaf (*H. petersiana*) phytoliths in Twe calculus. Women make palm leaf baskets and first chew the leaves to soften the fibers. Palm leaf phytoliths were only observed in three samples, all collected from women. This sample size is too small for statistical analysis, but this datum highlights the potential of the method to capture sex differences in behavior, as well as non-dietary processing of plants in the mouth.

3.2.2. Individual differences based on 'home' location

We also compared samples collected at Otjomuru/Okau with samples collected at Ohayuua. Dry season staple foods are different in these locations. The Twe eat palm nuts often at Ohayuua, but not at all at Otjomuru/Okau. *D. mespiliformis* fruits are a dry season staple at Otjomuru/Okau, but do not grow at Ohayuua. *D. mespiliformis* fruits do not contain starch or phytoliths, but palm fruits contain a high concentration of diagnostic phytoliths (refer to Table 3 for % dry weight). We found that dental calculus from people who live at Ohayuua is no more likely to preserve palm fruit phytoliths than calculus from people living at Otjomuru/Okau. Palm nut phytoliths were only found in seven of 74 samples (3 men, 4 women), and only one of these was collected from an individual living at Ohayuua. This finding likely reflects the level of mobility among the Twe, since they often leave their "home" camp and visit camps with different foods.

3.2.3. Intra-individual comparison of microfossil record

In order to assess how consistently microremains in dental calculus record diet across the population, we used a resampling approach to determine how similar any two randomly drawn individuals are in terms of the types of plants represented. Each person's plant representation was compared to every other person in the population to determine how many plants they share. We then calculated the mean number of plants shared across all individuals. We did this for subsets of the population with at least one plant, more than one, and more than two plants represented. Table 7 shows the mean number of plants shared by individuals in each subset.

Most samples do not share even a single plant. Looking only at the subset with two or more plants, there is still slightly less than one shared plant between samples. This lack of shared plants does not improve looking only within region or within sex. We would expect

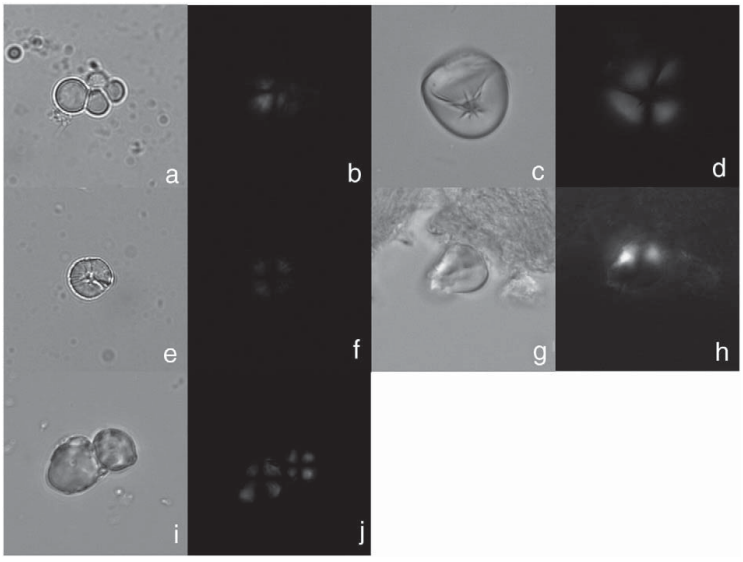

Fig. 4. Examples of identifiable starches recovered from Twe dental calculus. All images are shown at the same scale. Each box is 50 μm on a side. Images are paired; the first of each pair is shown under bright-field light and the second under cross-polarized light. a–b, *Adansonia digitata* L.; b–c, *Fockea angustifolia* K. Schum; d–e, *Pennisetum glaucum* L.; f–g, *Tylosema esculentum* A. Schreib; and h–i, *Zea mays* L.

Table 6
Regression models for dental calculus starch content.

Dependent variable	Intercept	Age		Sex		Sample weight		Total starches	
		B	SE B	B	SE B	SE	SE B	SE	SE B
Total starches	1.445	0.015*	0.006						
Total starches	1.792			0.564**	0.204				
Total starches	1.346	0.011	0.006	0.436*	0.203				
Total starches	2.030	−0.004	0.007	0.241	0.214	189.590*	69.949		
Total plants	0.078	0.002	0.010	−0.227	0.300	165.101*	65.005		
# starchy plants	−0.356	0.001	0.011	−0.156	0.348	179.600*	73.355		
# starchy plants	−0.862							0.054***	0.009

The dependent variable for each model is listed in the column on the left, with the independent variables following. The beta value (B) and standard error (SE B) for each model show the strength of each independent variable's effect. A missing value means that independent variable was not included in the model.
* $p < 0.05$.
** $p < 0.0$.
*** $p < 0.001$.

that most of these starches should come from the same plant, given the similarity in diet, but this was not the case. This large amount of individual variation in plant representation suggests that differences in plant representation do not necessarily reflect differences in plant consumption.

3.3. Are starches and phytoliths in Twe dental calculus a strong predictor of the range of plants consumed?

Microremain findings per individual are a poor predictor of the range of starch and phytolith producing plants consumed. Although nineteen Twe plant foods contain starch and phytoliths, the maximum number observed in an individual calculus sample is six. On a population level, starch grains and phytoliths are a better predictor of diet breadth. Across all 74 samples, starches and phytoliths from eleven different plant parts of ten plants (including two palm parts) are observed. Of these, six plants are represented by starches, and five plant parts of four plants are represented by phytoliths. We calculated the odds of finding any given starch producing plant in a given number of individual dental calculus samples by resampling from our data (iterated 50,000 times). Table 8 lists the number of individual samples needed to have 95% confidence of finding a given plant. Fig. 5 shows the same data in graphical form. In our simulation, some plants are visible at much smaller sample sizes than others, and for 95% confidence of viewing the full range of starchy plants represented in Twe calculus, our sample of 74 individuals is insufficient. We excluded phytolith producing plants from this analysis because very few calculus samples contained diagnostic phytoliths (18%).

Table 7
Similarity of plant representation in Twe calculus based on resampling from data.

Number of plants in calculus sample	Mean number of shared plants
1+	0.3996
2+	1.0583
3+	2.1333

Table 8
Number of individuals needed for 95% confidence of viewing each plant.

Plant	Required number of individuals
Adansonia digitata L.	Did not converge — 86% odds of finding with 75 people
Cucurbita sp.	Did not converge — 63% odds of finding with 75 people
Fockea angustifolia K. Schum	21
Pennisetum glaucum L.	16
Triticum sp.	55
Tylosema esculentum K. Schum	36
Zea mays L.	8

3.4. Is starch quantity in dental calculus samples proportional to dietary concentration?

We estimated the expected relative contribution of starches from each plant by multiplying the number of starches per gram dry weight by the average amount of each plant food consumed. On a population level, the relative dietary contribution of starch from each plant does predict the amount of observed starch in dental calculus ($B = 0.57$, $SE = 0.12$, $p = 0.0024$). However, the overwhelming majority of observed starch and expected starch come from maize. When maize is excluded from the analysis, the relationship between expected starch and observed starch disappears ($B = -1.51$, $SE = 1.28$, $p = 0.28$), suggesting that the relative representation of starch granules in dental calculus is not a good predictor of the dietary importance of plants with low starch content, or plants that are not intensively exploited.

4. Discussion

The identification of starch granules and phytoliths in Twe dental calculus gives an incomplete picture of Twe diet. This is in part because not all plants consumed by the Twe produce starches and phytoliths, but also because many of the starch and phytolith producing plants are not represented in calculus samples. There is a large amount of individual variation in the number of plants represented per calculus sample: from zero to a maximum of six. This maximum represents

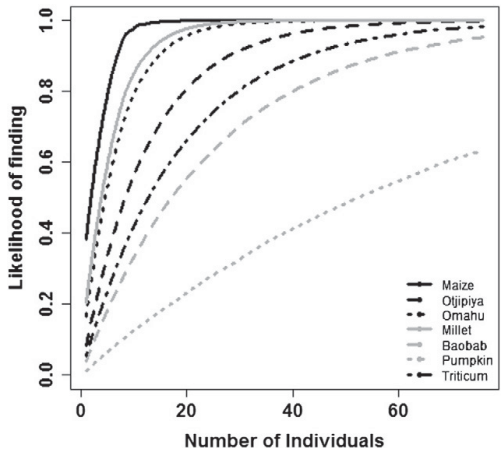

Fig. 5. Number of individuals needed for 95% confidence of viewing each plant.

fewer than one-quarter of the plants regularly consumed by the Twe, and fewer than one-third of the starch and phytolith producing plants in the Twe diet. These results suggest that dental calculus studies sampling a very small number of individuals are not representative of diet in larger populations.

On a population level, starches and phytoliths in Twe calculus are a better predictor of diet, with eleven plants represented, or one-third of the plants regularly consumed. However, the most commonly observed plant is maize (represented by starches), while other plants are represented at much lower frequencies. This indicates that the probability of observing many commonly eaten foods in dental calculus is very low. Resampling of our data from a probability perspective shows that a sample of 50 individuals or more is necessary to have 95% confidence in observing several plants. This has important implications for archaeological studies trying to identify the consumption of specific plants. Depending on the importance of the plant in the diet and its starch/phytolith content, a very large sample may be necessary.

The amount of variation between individuals suggests that comparisons of individuals' diets based on starches and phytoliths in dental calculus are problematic. Two randomly selected Twe with nearly identical diets may have a very different number and range of plants represented in their dental calculus (Table 7.). Population level comparisons of diet using large sample sizes are probably more appropriate for assessing differences in plant consumption between groups.

Our results indicate that the proportion of microremains from different plants is not correlated with the exploitation intensity of those plants. However, analysis of microfossils in calculus still speaks to interesting behavioral questions. For example, the presence of certain plants may point to changes in diet breadth. We observed several starch granules from *Tylosema esculentum* A. Schreib rhizomes in Twe calculus. This rhizome grows more than 0.5 m below hard packed, rocky soil, and takes considerable time and effort to extract. Once collected, the rhizome must be roasted for several hours, then pounded and peeled. Pending nutritional analysis, we suspect that this rhizome has a low caloric yield relative to the energetic and time costs of procurement and processing. This indicates that the Twe diet sometimes broadens to include resources with a low rate of energetic gain.

Though the majority of taxonomically identified starches in Twe calculus came from maize, we expected that maize starch would be more prevalent. Each participant reported eating maize at least once a day, every day, but maize starch was found only in 30% of the calculus samples. We expect that this may be in part due to processing. Maize is always ground and cooked, which causes significant damage and gelatinization to the starches, removing them from the calculus record.

The prevalence of maize starch in the Twe sample suggests a bias toward starch from domesticates in populations with mixed economies. Many plant domesticates are selected for high starch content. Maize has more starches per gram dry weight than all but two of the starchy wild foods in the Twe diet. These two starchy foods, baobab (*Adansonia digitata*) and *Lapeirousia* sp. (grass) corms are consumed regularly when in season, but are underrepresented in Twe calculus samples. Baobab starches were recovered from only two samples, and *Lapeirousia* starches were absent from all samples. Although these foods are not consumed at the same frequency year-round as maize, given their high starch content we expected to observe them more frequently in Twe calculus samples. It is unlikely that processing or cooking removed these starches from the calculus record. Baobab is eaten raw and *Lapeirousia* corms are cooked on hot coals only long enough to loosen the tunics (~2–5 min), which does not damage the starch. The size and features of these starches may have affected their preservation in the record or our ability to recognize them. Baobab starches are very small and difficult to confidently identify. However *Lapeirousia* starches are larger and more distinctive, so it is unlikely that we failed to recognize them. It may be that *Lapeirousia* starches are more affected by salivary enzymes or more easily damaged during HCl processing, but we have not assessed this.

The starchy wild plants that are most regularly consumed are *Fockea angustifolia* K. Schum and *Ximenia americana* L. *F. angustifolia* tubers have a moderate starch content and are probably an important part of the Twe diet. We suspect that *F. angustifolia* consumption is underreported due to a stigma against digging for food, but all Twe in our sample admit eating it whenever food stores run low. *F. angustifolia* is relatively common in Twe calculus (identified in 14% of Twe calculi), but compared with maize is probably underrepresented (23% of samples). Seasonally, *X. americana* fruits are eaten as regularly as maize flour, but with only 27 starches per gram dry weight, they are much less likely to contribute starches to the calculus record, and we did not observe any *X. americana* starches in our sample. Like baobab starches, *X. americana* starches are very small, and this may make them more difficult to identify than starches from domesticates.

Perry (2002) shows that starches from manioc and sweet potato are systematically larger than wild predecessors, and Piperno et al. (2009) show that maize has a higher starch content and larger starch granules than wild teosinte varieties. Due to a high starch content, many domesticates are probably more likely to contribute to the dental calculus microfossil record. Additionally, large diagnostic starch granules may mean that domesticates are more easily identified in dental calculus. There are several wild foods in the Twe diet that are as important as maize flour in different seasons (e.g. *B. discolor* berries in the rainy season and *D. mespiliformis* fruits in the dry season), but these plants contain neither starches nor phytoliths, and are invisible in dental calculus.

5. Conclusions

We suggest that analysis of starch granules and phytoliths in dental calculus is best suited to questions about the presence or absence of specific plants in the diet, keeping in mind the sample size. This method probably does not address 'bigger picture' questions about diet such as exploitation intensity, or inter-individual differences in the range of plants consumed. Starches and phytoliths in dental calculus have the potential to identify specific plant taxa consumed. In this way, this method provides a complement to other methods of plant–diet reconstruction such as stable isotope analysis and tooth macro-and microwear studies that give information about the categories of plants consumed or mechanical properties of those plants. The ability to identify the consumption of specific plants is both important and exciting.

This work represents a first attempt to understand how diet is recorded in dental calculus. However, we do not address dental calculus formation and the mechanism of starch and phytolith incorporation. The dental science literature highlights individual variation in calculus formation, and our results suggest that the factors controlling this variation also contribute to individual variation in starch and phytolith preservation. We also suspect that other analytical methods may complement microscopic analysis of dental calculus, leading to a fuller understanding of ancient diet and lifeways. These include pyrolysis of dental calculus and proteomic analysis (cf. Buckley et al., 2014; Hardy et al., 2012; Warinner et al., 2014).

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CHAPTER 5

CONCLUSION

The link between diet, morphology, and behavior in the animal kingdom is well documented. Among nonhuman primates, diet is arguably predictive of brain size and intelligence, underlies patterning in social systems, and determines other important behavioral attributes. Changes in diet are an assumed selective pressure for both morphological and behavioral changes in the hominin lineage. As such, diet reconstruction is a major focus in paleoanthropology. Our current understanding of hominin diet is informed by both archaeological evidence and ethnographic observations of living foragers. Despite the long trajectory of research, we still know surprisingly little about the changes in diet that underlie novel adaptations in the hominin lineage.

This dissertation challenges traditional wisdom that the inclusion of hunted large game was the key dietary change that supported the evolution of distinctly human morphological, life history, and behavioral traits. Until now, the nutritional qualities of plant foods included in forager diets has been under-explored. Most anthropological studies of plant food nutritional composition focus on plant underground storage organs, which are characterized in the literature as an alternative to animal foods in selecting for morphological and behavioral changes in the hominin lineage.

The second chapter of this dissertation attempts to broaden the focus on the potential importance of plant foods in human evolution. In this chapter, my coauthors and I present data on the nutrient composition and amino acid content of common fruits and underground storage organs included in the Twe diet. We discuss the nutritional contributions of both fruits and underground storage organs, and conclude that the importance of animal foods to hominin diets is overstated. Our data suggest that some animal input is necessary for the Twe diet, but that contribution can come from a variety of animal sources. We argue that the proposed importance of large game hunting in human evolution was not nutritional.

The third chapter explores the nutritional qualities of one resource type, plant underground storage organs, and discusses energy expenditure during plant procurement.

Underground storage organs are the only plant food that have received attention in the literature, and their inclusion in hominin diets may have supported the evolution of human life histories and the spread of hominins out of Africa. However, opinion is divided on whether USOs can provide adequate nutrition for large bodied hominins. We find that USOs vary dramatically in their caloric content, but are generally high in carbohydrate and low in fiber. We test the assumption that return rates during USO foraging are adequate to provision a forager and at least one dependent by examining energy expenditure while digging. We find that some USOs require substantial effort to procure, which drastically decreases the overall (net) caloric return rate. In some cases, net return rates remain high, but in others, the rates are very low, indicating that these USOs may not be an attractive resource relative to other available resource types.

The final chapter explores the archaeological record of plant consumption preserved in dental calculus. While this method of diet reconstruction has become popular in the last decade, it is unknown how reliably the plant microremains trapped in dental calculus record plant consumption. We compare data on Twe plant consumption with the starch granules and phytoliths in Twe dental calculus and find that the microremain record does reflect plant consumption, but in a limited sense. Most of the plants consumed are not recorded in dental calculus, and there is a large amount of individual variation in both the number and types of plants recorded, despite remarkable similarities in diet across our sample. We conclude that the method is most useful in answering questions about the diet of populations, and is not appropriate for observing individual differences.

APPENDIX A

FULL METHODS FOR NUTRITIONAL ANALYSIS

Dried plant probes were processed in a grinding mill (Cyclotec 1093, FOSS TECATOR AB, Hgans, Sweden) at 1 mm screen sieve and analyzed by Standard chemical methods according to descriptions of the VDLUFA (Verband deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten), Methodenhandbuch Vol. III [1] to determine nutrient content. Samples were assayed for the contents of dry matter (DM), crude ash (Cash), crude protein (CP), crude fat (Cfa), crude fiber (Cfe), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and other constituents as described below.

All measurements were performed in duplicate and chemical values are reported as percentage of dry matter. The evaluation of the total energy content is based on conventional estimates of the energy values of different nutrients. The estimation of metabolizable energy follows Conklin-Brittain et al. [2] and assumes some fiber fermentation in the gut.

$$\text{ME kcal/100 g OM } \bar{(}4 \ \ \%\text{TNC}) + (4 \text{ x } \%\text{CP}) + (9 \ \ \%\text{lipid}) + (1.6 \ \ \%\text{NDF}) \qquad \text{(A.1)}$$

where ME=metabolizable energy, OM=original matter, TNC=total nonstructural carbohydrate, CP=crude protein, and NDF=neutral detergent fiber.

Fresh weight was calculated using the following equation form Conklin-Brittain et al. [2]:

$$\text{Energy content of a fresh food} = (\text{ME}/\ 100 \text{ g OM}) \ \ (\text{g OM}/\ \text{g of the fresh food}) \qquad \text{(A.2)}$$

All chemicals used were either technical grade or HPLC grade for the chromatographic analysis. Compounds were obtained from ROTH(Karlsruhe, Germany) unless noted otherwise.

Dry matter was determined after drying the samples at 105C for 4 h, and crude ash after 7 h of combustion at 600C in a muffle furnace.

Crude fiber (Rfe) content was analyzed as described in the AOAC [1] using the FibertecTM system (Hot Extractor 1020; FOSS TECATOR AB, Hgans, Sweden). Crude fiber was obtained from the loss in weight on incineration of dried residue remaining after the consecutive treatment of the probes with both 1.25 % sulfuric acid and 1.25% potassium under specific conditions. Samples with more than 2 % fat content were extracted with petroleum ether before crude fiber determination.

For determining the ADF, ADL and NDF content of the plant samples according to the methods of van Soest et al. ([3]), we used a modified filter bag technique by ANKOM technology (ANKOM Technology Corporation, NY, USA, method 5 and 6 4/13/111). 0.45-0.55 g of grinded probe material was weighed into nitrogen and ash free filter bags and then subjected to extraction in the ANKOM 200 fiber Analyzer. After extraction, bags were washed with hot distilled water and acetone, tried, and weighed back. ADL was received by the acidic treatment (72 % H2SO4) of the filter bags after performing ADF determination of sample material. Hemicellulose was determined as the difference of NDF and ADF, whereas cellulose was calculated as the difference of ADF-ADL [4].

The Soxlet extraction method [5] was used to measure total fat content of the different plant materials. Following an initial acid hydrolysis procedure (4 mol/l HCL, 30 min), crude fat was extracted for 2 h with petroleum ether (40C-60C) in SOXTECTM 2050 autoextraction Unit (FOSS, Rellingen).

Total nitrogen content was estimated via a standard semimicro Kjeldahl method [6] on a Kejeltec 1030 auto analyzer (FOSS TECATOR AB, Hgans, Schweden). All samples (0.2 g) were digested for 1.5 h in a solvent mixture consisting of 4 ml concentrated sulfuric acid (96%) and one copper containing catalyst tablet (31.5 g K2SO4 , 0.15 g CuSO4 $*$5H2O, FOSS, Rellingen) to oxidize the organic substance. Subsequently, the solvents were diluted with 15 ml of distilled water, distillated with a small amount of sodium hydroxid (32 %) into 30 ml of 1% boric acid, and finally back titrated with 0.1 mol/l HCl. Crude protein content was calculated as N$*$6.25.

The nitrogen free extractives (NfE), representing -glycosidically bonded polysaccharides, soluble carbohydrates as well as soluble parts of cellulose, hemicellulose, lignin, and pectin were calculate by subtracting the contents of crude ash, crude protein, crude fat, and crude fiber from dry matter:

$$\text{NfE} = \text{Dry matter} \quad (\text{Cash} + \text{Cp} + \text{Cfa} + \text{Cfe}) \tag{A.3}$$

A UV- based gradient reversed phase HPLC method was used to determine and quantify the amino acid composition of the plant material. In preparation of the chromatographic analysis, dried samples, containing 7 to 10 mg nitrogen each, were first hydrolyzed in 6N HCl and subsequently derivatized with phenylisothiocyanate (PITC) following a precolumn derivatization procedure as described in Elkin and Wasynczuk [7]. To also determine the hydrolytically labile amino acids methionine and cysteine, additional aliquots of each plant probe had to be oxidized with phenolic formic acid prior to the hydrolisation and derivatization. The accuracy of the used HPLC method was evaluated using different external and internal standards. The reference standard solution (SIGMA-ALDRICH, Taufkirchen, Germany) contains 18 different L-amino acids in a concentration of 2.5 mol/ml as well as L-cystein with 1.25 mol/l. In addition, 2.5 mol/ml each of methionine sulphone, and cysteic acid were added. 2.5 mol/ml of norleucin was used as the internal standard.

A automated waters HPLC system (WATERS GmbH, Eschborn, Germany) containing a 150∗4,6 mm ODS-Hypersil RP-column (5m) and a 10∗4.6 mm ODS guard column (5 m) (both ALTMANN-ANALYTIC, Mnchen, Germany) as solid phase was used to separate, under a constant temperature of 30C, the derivatized amino acids and appropriate standards by gradient elution according to the procedure shown in Table A.1. The single amino acids were detected by UV at 254 nm based on the retention time determined for the individual reference amino acids under defined experimental conditions. Amino acid content was quantified and normalized by the aid of the external and internal amino acid standard. Calculation was based on the area under peak established for a given amino acid of a known standard concentration.

Table A.1: Gradient program for the separation of PITC derivatized amino acids[a]

Time (min)	Flow rate (ml/min)	% Buffer A[b]	% Buffer B[c]	Gradient curve[d]
Initial	1.6	100	0,0	
3	1.7	95,1	4,9	2
24	1.7	97,0	3,0	2
26	1.7	89,0	11,0	2
28	1.7	74,0	26,0	2
43	1.7	64	36,0	2
46	1.7	57	43,0	10
49	1,7	50,0	50	2
50	1,7	0,0	100	4
54	1,7	0,0	100	2
56	1,7	100	0,0	8
61	1,6	100	0,0	2

[a] Column temperature maintained at 30 C
[b] 0.05 M sodium acetate in water, pH 7,2)
[c] (0.1 M sodium acetate, acetonitrile, water (46:44:10 v/v/v)
[d] curve 2 , 4 (hold), 8 (recalibration) and 10 are non linear

A.1 References

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APPENDIX B

AMINO ACID SPECTRA FOR TWE PLANT FOODS

Table B.1: Amino acid content of Twe plant foods, mg/100g dry plant material

Taxon	Cysteine	Methionine	Aspasparic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Valine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine	Tryptophan
B. discolor	59.48	25.506	391.962	96.89	128.376	562.369	84.782	137.186	132.311	134.904	82.475	183.976	99.973	97.136	46.21	92.589	85.341	10.58
D. mespiliformis	31.025	24.037	143.859	87.974	86.882	57.293	131.658	104.632	95.661	110.762	66.955	141.576	30.954	77.527	50.87	137.266	83.271	3.782
H. Petersiana	42.586	29.132	265.292	127.584	178.398	264.842	129.936	149.552	176.474	173.62	104.48	235.437	134.575	126.104	52.58	180.082	150.907	6.7
F. angustifolia	58	30.691	675.126	125.967	173.724	451.88	89.307	132.751	159.981	132.941	86.579	137.719	56.047	84.213	71.821	150.865	139.641	0.33
Lapeirousia sp.	168.504	52.927	342.643	195.576	202.416	751.28	275.392	163.738	207.987	223.569	125.476	307.229	239.577	125.705	117.5	215.528	648.87	2.177
Camptorrhiza sp.	190.819	168.558	894.16	321.012	427.656	751.279	710.646	382.16	409.025	418.959	236.742	479.836	238.477	386.785	172.583	222.382	854.067	20.25
F. sycomorus	62.992	80.144	569.771	276.928	354.59	734.184	251.467	342.468	319.735	387.041	248.133	507.071	199.832	296.859	140.088	291.691	419.087	13.532
T. esculentum	70.862	51.362	5611.664	134.275	174.882	321.257	383.217	112.6	155.591	274.423	118.46	170.671	150.168	121.597	214.945	254.768	263.299	6.27
P. glaucum	168.865	227.464	842.299	389.995	503.212	1801.168	411.092	317.359	808.207	668.035	380.106	1129.655	268.134	491.007	322.61	315.351	452.493	41.339
Z. mays[a]	213.228	278.199	960.693	445.719	611.613	1936.901	753.229	420.84	872.298	641.105	345.719	1437.662	338.28	544.687	267.64	481.341	454.774	17.308
Z. mays[b]	190.388	270.913	484.831	224.982	433.15	1456.95	598.502	294.15	635.64	430.341	227.459	1167.56	248.28	398.797	216.34	229.461	372.288	9.363

[a]Twe gardens
[b]government